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ABSTRACT:

The present invention provides polynucleotide and amino acid sequences which encode and identify a novel human myeloid terminal differentiation response gene designated MYD118. The present invention also provides for myd118 antisense molecules. The invention further provides genetically engineered expression vectors and host cells for the production of purified MYD118 polypeptide; antibodies, antagonists and inhibitors of MYD118 polypeptide; and pharmaceutical compositions and methods of treatment based on polynucleotide sequences encoding MYD118 and MYD118 polypeptide. The invention specifically provides for use of the myd118 polynucleotide sequences as a diagnostic composition for the detection of myeloproliferative diseases and leukemias. The invention also relates to therapeutic methods and compositions based upon the nucleotide sequences for myd118. The invention further provides antibodies which specifically bind to MYD118.



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(57) Abstract

The present invention provides polynucleotide and amino acid sequences which encode and identify a novel human myeloid terminal differentiation response gene designated MYD118. The present invention also provides for MYD118 antisense molecules. The invention further provides genetically engineered expression vectors and host cells for the production of purified MYD118 polypeptide; antibodies, antagonists and inhibitors of MYD118 polypeptide; and pharmaceutical compositions and methods of treatment based on polynucleotide sequences encoding MYD118 polypeptide. The invention specifically provides for use of the MYD118 polynucleotide sequences as a diagnostic composition for the detection of myeloproliferative diseases and leukemias. The invention also relates to therapeutic methods and compositions based upon the nucleotide and amino acid sequences for MYD118. The invention further provides antibodies which specifically bind to MYD118.

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5' C GGA CTA CCG TTG GTT TCC GCA ACT TCT TGG ATT ATC CTC GCC AAG GAC TTT
63 72 81 90 99 108
GGA ATA TAT TTT TCC GCC TTT TCT GGA AGG ATT TCG CTT CCC GAA GGT CTT
117 126 135 144 153 162
GGA CGA GCG CTC TAG CTC TGT GGG AAG GTT TNG GGC TCT CTG GCT CCG ATT TTG
171 180 189 198 207 216
GAA TTT CTC CCG GGG GAC TGC COT GGA GCC GCA TCC ACT GTG GAT TAT AAT TGC
225 234 243 252 261 270
AAC ATG ACG CTG GAA GAG CTC GTG GCG TGC GAC AAC GCG GCG CAG AAG ATG CAG
N T L E E L V A C D N A A Q K H Q
279 288 297 306 315 324
ACG GTG ACC GCC GCG GTG GAG GAG CTT TTG GTG GCC OCT CAG CCG CAG GAT CCG
T V T A A V E E L L V A A Q R Q D R
333 342 351 360 369 378
CTC ACA GTG GGG GTG TAC GAG TCG GCC AAG TTG ATG AAT GTG GAC CCA GAC AOC
L T V G V Y E E A K L N N V D P D B
387 396 405 414 423 432
GTG GTC CTC TCC TTT GCC ATT AAC GAG GAG GAG GAT GAC ATC GCC CTG
V V L C L L A I N E E E B D D I A L
441 450 459 468 477 486
CAA ATC CAC TTC ACG CTC ATC CAG TCC TTC TGT AAC AAC GAC ATC AAC ATC
Q I H F T L I Q S F S C N N D I N I
495 504 513 522 531 540
GTG CCG GTT TCG GGC ATG CAG CCG CTG GCG CAG CTC CTG GGA GAG CCG GCG GAG
V R V S G N Q R L A Q L L O E P A E
549 558 567 576 585 594
ACC CAG GGC ACC ACC GAG GCC GGA GAC CTG CAT TGT CTC CTG GTC ACG AAC CCG
T Q G T T E A R D L B C L L V T N F
603 612 621 630 639 648
CAC ACG GAC GCC CCG AAG AGC CAC GGC TTG GTG GAG GTG GCC AGC TAC TGC GAA
H T D A R K S H G L V E V A B Y C E
657 666 675 684 693 702
GAA ACG CCG GGC AAC AAC CAG TGG GTC CCC TAC ATC TCT CTT CAG GAA CCG TGA
E S R G N N Q W V P Y I S L Q E R
711 720 729 738 747
GGC CTT CCC AGC AGC AGA ATC TGT TTG AAT TGC TGC CAC AAC CAA 3'

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NOVEL HUMAN MYELOID TERMINAL DIFFERENTIATION RESPONSE GENE**RELATED APPLICATIONS**

The present invention is a continuation in part application of United States Application
5 Serial Number 08/602,208 filed February 15, 1996, which is incorporated herein by reference in its entirety.

TECHNICAL FIELD

The present invention relates to nucleic acid and amino acid sequences of a novel human
10 myeloid terminal differentiation response gene found in cDNA libraries made from human fetal liver/spleen tissue and non-adherent peripheral blood mononuclear cells and to the use of these sequences in the diagnosis, study and treatment of disease.

BACKGROUND ART

15 Cell numbers are regulated by a balance among proliferation, growth arrest and programmed cell death (apoptosis; Fornace AJ et al. 1992 Ann New York Acad Sci 663:139-153). Genes induced by various growth arrest and apoptotic stimuli are the tumor suppressor gene p53, myeloid differentiation primary response genes (MyD genes), and growth arrest and DNA damage inducible genes (GADD genes; Selvakumaran M et al. 1994 Mol Cell Biol
20 14:2352-60).

Animal cells respond to differentiation signals which turn on or off the appropriate genes resulting in conversion of proliferating, undifferentiated cells into nonproliferating, highly specialized, differentiated cells. An example of this process is the differentiation of myeloid precursor cells into mature granulocytes and macrophages. Blocks in the differentiation process
25 appear to be a major step in tumor progression, and lesions in genes involved in terminal differentiation contribute to the development of malignant tumors (Liebermann MA et al. 1994 Stem Cells 12:352-69).

Liebermann et al. suggest that MyD genes function as positive regulators of terminal hematopoietic cell differentiation, which is associated with inhibition of cell growth and
30 apoptosis. Selvakumaran et al. supra provide evidence that MyD family member, murine MYD118, described as a terminal differentiation response gene, is expressed in M1D+ myeloid precursor cells following induction of terminal differentiation and growth arrest by IL6 and has

been shown to be a positive regulator of apoptosis induced by TGF β 1. Additionally, leucine zipper transcription factors of the fos/jun family have been identified as MyD genes, specifically MyD21, MyD42 and MyD63, and function as positive regulators of hematopoietic cell differentiation, increasing the differentiation of myeloblastic leukemia cells in vitro and reducing the aggressiveness of the leukemic phenotype in nude mice. Liebermann et al. supra suggest that lesions in the MyD genes of the fos/jun family that affect expression or function of the genes contribute to development of leukemias.

The cDNA sequence and deduced amino acid sequence of murine MYD118 is disclosed in Abdollahi A et al. (1991 Oncogene 6:165-17) who indicate that the cDNA nucleotide sequence of murine MYD118 predicts a protein of 160 amino acids, which does not contain protein secretory signals, transmembrane domains or known protein-DNA binding motifs, but does appear to contain a protein kinase phosphorylation site at position 204, two casein kinase II phosphorylation sites at positions 215 and 231 and several AT₃ motifs in its 3' untranslated region. Abdollahi et al. observed detectable levels of MYD118 RNA in myeloid precursor enriched murine bone marrow, but not in several other non-myeloid murine tissues, such as liver or brain. Abdollahi et al. also observed that MYD118 expression was induced in the absence of protein synthesis, following stimulation of M1D+ cells by IL-1, LPS and leukemia inhibitory factor (LIF).

The amino acid sequence for murine MYD118 is 75% similar (57% identical) to the amino acid sequence for murine GADD gene, GADD45, which is regulated in part by the tumor suppressor gene p53 (Zhan Q et al. (1994) Cancer Res 54: 2755-60; Carrier F et al. (1994) J Biol Chem 269:32672-32677). GADD and MYD118 are two separate but closely related genes and act synergistically to suppress growth of hematopoietic cell lineages.

Various portions of the nucleotide sequence encoding human MYD118 have been disclosed in GenBank 92 in cDNA libraries made from tissue from hippocampus (M77995), retina (H84533 and H83991), olfactory epithelium (H71592), human fetal lung (D310470 and D31559), breast (H44355, R55161 and R82994), placenta (R24009, R63425, R21918 and R22497), human white blood cells (T33963), human brain (T35368), human pancreas (T29941), liver (T40088), prostate gland (T35225) and lung (T35563). The complete nucleotide sequence encoding human MYD118 has not been disclosed.

Myeloproliferative diseases and leukemias are neoplasms of the hematopoietic stem cell and include acute lymphocytic leukemia (ALL), acute myelogenous leukemia (AML), chronic

lymphocytic leukemia (CLL), hairy cell leukemia and chronic myelogenous leukemia (CML); polycythemia vera (PV); agnogenic myeloid metaplasia with myelofibrosis (AMM/MF); and essential thrombocytosis (ET). Research suggests that the myeloproliferative diseases arise as clonal expansions of a single transformed stem cell and that all of the myeloid cells of the blood are derived from the neoplastic clone. In leukemia, leukemic cells proliferate primarily in the bone marrow and lymphoid tissues and are characterized according to the cell type involved (myeloid or lymphoid). Acute leukemia is characterized by proliferation of immature myeloid or lymphoid cells. CLL is a hematologic neoplasm characterized by the accumulation of mature-appearing lymphocytes in the peripheral blood associated with infiltration of the bone marrow.

Hairy cell leukemia is characterized by peripheral blood cytopenias, splenomegaly and malignant cells in the blood and bone marrow. CML is characterized by marked splenomegaly and the production of increased numbers of granulocytes, particularly neutrophils, in the marrow and blood. PV is characterized by splenomegaly and an increased production of all myeloid elements, but is dominated by an elevated hemoglobin concentration. AMM/MF is characterized by the tendency of the neoplastic stem cells to lodge and grow in multiple sites outside the marrow, progressive splenomegaly, the gradual replacement of marrow elements by fibrosis, and variable changes in the number of granulocytes and platelets. ET is characterized by an elevated platelet count and represents the overproduction of platelets in the absence of a recognizable stimulus. In cultures of bone marrow cells from individuals subject to ET, colonies of megakaryocytes from megakaryocyte progenitors form in the absence of added stimulus, whereas such colonies do not occur with marrow cell cultures from normal individuals. (Braunwald et al. (Eds, 1987) Harrison's Principles of Internal Medicine, 11th ed. McGraw-Hill, New York NY).

DISCLOSURE OF THE INVENTION

The present invention relates to human MYD118 whose nucleic acid sequence has been identified among the polynucleotide sequences of cDNA libraries made from human fetal liver-spleen tissue and non-adherent peripheral blood mononuclear cells and to the use of the nucleic acid and amino acid sequences of MYD118 in the study, diagnosis and treatment of disease states related to proliferation, specifically myeloproliferative diseases and leukemias.

The nucleic acid sequence of myd118 (SEQ ID NO:1) and the protein it encodes, MYD118 (SEQ ID NO:2) is disclosed herein in Figure 1. The amino acid homology among human MYD118, murine MYD118 (SEQ ID NO:3) and human GADD 45 (SEQ ID NO:4) is

shown in Figure 2.

The present invention is based in part on the amino acid homology that human MYD118 shares with murine MYD118 and the ability of murine MYD118 and other MYD family members to stimulate terminal differentiation of hematopoietic cells, to arrest cell growth and to modulate the leukemic phenotype *in vivo*. The present invention is also based in part on the presence of nucleic acid sequences encoding MYD118 in a cDNA library made from fetal liver/spleen tissue and non-adherent peripheral blood mononuclear cells, where hematopoietic cells would be expected to be found. Nucleic acid sequences encoding MYD118 are not detected in samples of cDNA libraries made from malignant hematopoietic cells sources where expression of genes related to cell growth arrest and apoptosis may be absent, aberrant, deleted or expressed at low levels.

Therefore, expression of human MYD118 may be altered, absent or at low levels in individuals subject to myeloproliferative disease and leukemias. Human MYD118, and nucleic acid sequences that encode it and oligonucleotides, peptide nucleic acid (PNA), fragments, portions or antisense molecules thereof, provide the basis for diagnostic methods for the early and accurate detection and/or quantitation of MYD118 associated with abnormally proliferating hematopoietic cells such as, myeloproliferative diseases and leukemias, including but not limited to acute lymphocytic leukemia (ALL), acute myelogenous leukemia (AML), chronic lymphocytic leukemia (CLL), hairy cell leukemia, chronic myelogenous leukemia (CML); polycythemia vera (PV); agnogenic myeloid metaplasia with myelofibrosis (AMM/MF); and essential thrombocytosis (ET). For example, the nucleotide sequence for myd118 disclosed herein, or fragments thereof, may be used in hybridization assays of biopsied cells or tissues or bodily fluids to diagnose abnormalities in myd118 gene expression in individuals subject to or at risk for a myeloproliferative disease or leukemia. Myd118 gene expression may be present at low levels, entirely absent or altered in such a disease states. Additionally, there may be a chromosomal aberration, such as a deletion or mutation, present in the region of the gene encoding MYD118 in disease states related to proliferation of hematopoietic cells.

The nucleotide and amino acid sequences for MYD118 may also be used in the diagnosis and treatment of other disease states related to abnormal proliferation of cells associated with severe inflammation, such as rheumatoid arthritis, psoriasis (characterized by proliferation of epidermal cells) and lymphomatoid granulomatosis (characterized as a lymphoproliferative disease) where it would be desirable to terminally differentiate proliferating cells and arrest their

growth.

Accordingly, the present invention provides diagnostic compositions and diagnostic tests for the detection of myd118 nucleotide sequences in biological samples. Such a diagnostic test comprises the steps of combining the biological sample with a first nucleotide sequence which
5 comprises an myd118 nucleotide sequence, or a non-conserved fragment thereof, under conditions suitable for the formation of a nucleic acid hybridization complex; detecting said hybridization complex, wherein the presence of said complex correlates with the presence of a second nucleotide sequence comprising myd118 nucleotide sequences in said biological sample; and comparing the amount of the second nucleotide sequence in said sample with a standard
10 thereby determining whether the amount of said second nucleotide sequence varies from said standard, wherein the presence of an abnormal level of said second nucleotide sequence correlates positively with a myeloproliferative disease. An abnormal level of nucleotide sequences encoding MYD118 in a biological sample may reflect a chromosomal aberration, such as a nucleic acid deletion or mutation. Accordingly, nucleotide sequences encoding MYD118
15 provide the basis for probes which can be used diagnostically to detect chromosomal aberrations such as deletions, mutations or chromosomal translocations in the gene encoding MYD118.

The present invention also provides a diagnostic test for the detection of myd118 nucleotide sequences in a biological sample, comprising the steps of combining the biological sample with polymerase chain reaction primers under conditions suitable for nucleic acid
20 amplification, wherein said primers comprise non-conserved fragments of the nucleotide sequence of SEQ ID NO:1, detecting amplified nucleotide sequences, and comparing the amount of amplified nucleotide sequences in said biological sample with a standard thereby determining whether the amount of said nucleotide sequence varies from said standard, wherein the presence of an abnormal level of said nucleotide sequence correlates positively with a myeloproliferative
25 disease.

Additionally, human MYD118 and the nucleic acid sequences that encode it will provide the basis for pharmaceutical compositions for the treatment of myeloproliferative diseases, such as ALL, AML, CLL, hairy cell leukemia, CML, PV, AMM/MF and ET. For example, nucleotide sequences that encode MYD118 can be administered alone or in combination with
30 nucleotide sequences encoding tumor suppressor genes, such as p53 (known to regulate the expression of related family member GADD45), p16 and p21, through gene therapy techniques to individuals with CML to induce terminal differentiation of granulocytes, thereby arresting

leukemic cell proliferation.

Alternatively, myd118 nucleic acid antisense molecules or antagonists of MYD118 protein may be used to block the activity of human MYD118 in conditions where it would be preferable to block positive regulators of cell growth arrest. For example, MYD118 may be used
5 alone or in combination with other agents in the ex vivo culturing of hematopoietic stem cells intended for autologous transplant to individuals lacking cells of the hematopoietic lineage such as, for example, individuals subject to HIV infection or individuals who have undergone chemotherapy or radiation therapy.

The present invention also relates, in part, to expression vectors and host cells comprising
10 polynucleotide sequences encoding MYD118 for the in vivo or in vitro production of MYD118 protein.

Additionally, the present invention relates to the use of MYD118 polypeptides, or fragments or variants thereof, to produce anti-MYD118 antibodies and to screen for antagonists or inhibitors of MYD118 polypeptides which can be used diagnostically to detect and quantitate
15 MYD118 protein levels in disease states related to proliferation.

The present invention further relates to methods of treating individuals subject to myeloproliferative disease comprising administering compositions comprising purified MYD118 polypeptides or variants thereof, to subjects at risk for or having myeloproliferative disease or leukemia.

20 The present invention also relates to pharmaceutical compositions comprising effective amounts of MYD118 protein or nucleic acid encoding MYD118 for the treatment of myeloproliferative diseases or leukemias.

The present invention also encompasses the use of gene therapy methods for the introduction of nucleotide sequences encoding MYD118 into individuals having or at risk for
25 myeloproliferative diseases, or leukemias.

The invention further provides diagnostic assays and kits for the detection of MYD118 in cells and tissues comprising purified MYD118 which may be used as a positive control, and anti-MYD118 antibodies. Such antibodies may be used in solution-based, membrane-based, or tissue-based technologies to detect any disease state or condition related to the expression of
30 MYD118 protein or expression of deletions or variants thereof.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 displays the polynucleotide (SEQ ID NO:1) and deduced amino acid (SEQ ID NO:2) sequence for MyD118. Sequences shown in this Figure were produced using the multisequence alignment program of DNASTAR software (DNASTAR Inc, Madison WI). SEQ ID NO:1 also contains untranslated 5' and 3' regions of myd118.

5 Figure 2 displays the amino acid alignment of human MYD118 (SEQ ID NO:2), murine MYD118 (SEQ ID NO:3) and human GADD45 (SEQ ID NO: 4). Boxed residues match the consensus sequence exactly.

Figure 3 displays an analysis of the hydrophobicity and other characteristics of MYD118 based on the predicted amino acid sequence (produced using DNASTAR software).

10

MODES FOR CARRYING OUT THE INVENTION

The present invention relates to human MYD118 and to the use of the nucleic acid and amino acid sequences of MYD118 in the study, diagnosis and treatment of disease states, specifically myeloproliferative diseases and leukemias.

15 The present invention is based in part on the amino acid homology that human MYD118 shares with murine MYD118 and the ability of murine MYD118 and other MYD family members to stimulate terminal differentiation of hematopoietic cells, to arrest cell growth and to reduce a leukemic phenotype *in vivo*. The present invention is also based in part on the presence of nucleic acid sequences encoding MYD118 in a cDNA library made from fetal liver/spleen
20 tissue and non-adherent peripheral blood mononuclear cells, where hematopoietic cells would be expected to be found. Nucleic acid sequences encoding MYD118 were not detected in randomly selected samples of about 500 to 5700 usable sequences in cDNA libraries made from THP-1 cells, the human promonocyte line derived from the peripheral blood of an individual subject to acute monocytic leukemia (ATCC accession TIB 202; INCYTE libraries: THP1NOB01,
25 THP1PEB01, THP1PLB01 and THP1PLB02); U937 cells, made from malignant cells from the pleural effusion of an individual subject to histiocytic lymphoma (ATCC accession CRL 1593; Sundstrom C and K Nilsson (1976) Int J Cancer 17:565-577; INCYTE library U937NOT01); T/B lymphoblasts from a leukemia source (Stratagene (La Jolla, CA), STR 937214; INCYTE library TBLYNOT01); peripheral blood white blood cells from an individual with myelogenous
30 leukemia (INCYTE library AMLBNOT01); and a human mast cell line from an individual subject to mast cell leukemia (INCYTE library HMCINOT01) where expression of genes related to cell growth arrest and apoptosis may be absent or aberrant. Nucleic acid sequences encoding

MYD118 were not detected in a cDNA library from peripheral blood granulocytes (INCYTE library NEUTFMT01); pooled bone marrow samples (INCYTE library BMARNOR02); ataxia telangiectasia fibroblast cell line (INCYTE libraries: FIBRAGT01, FIBRAGT02 and FIBRANT01); or adult spleen (INCYTE library SPLNNOT02). MYD118 related family member, GADD45, is known to be absent in individuals subject to ataxia telangiectasia (Zhan et al. (1994) Mol and Cell Biol 14:2361-2371).

Therefore, expression of human MYD118 may be altered, absent or not detected in individuals subject to myeloproliferative disease and leukemias. Additionally, the gene encoding MYD118 may be involved in a chromosomal aberration such as a deletion, mutation ie, a point or internal mutation, translocation or may contain tri-nucleotide repeats known to be present in chromosomal abnormalities. Human MYD118, nucleic acid sequences that encode MYD118 and oligonucleotides, peptide nucleic acid (PNA), fragments, portions or antisense molecules thereof, provide the basis for diagnostic methods for the early and accurate detection and/or quantitation of MYD118 associated with myeloproliferative diseases and leukemias, such as acute lymphocytic leukemia (ALL), acute myelogenous leukemia (AML), chronic lymphocytic leukemia (CLL), hairy cell leukemia, chronic myelogenous leukemia (CML); polycythemia vera (PV); agnogenic myeloid metaplasia with myelofibrosis (AMM/MF); and essential thrombocytosis (ET). For example, the nucleotide sequence for myd118 disclosed herein, or fragments thereof, may be used in hybridization assays of biopsied cells or tissues to diagnose abnormalities in myd118 gene expression in individuals with or at risk for a myeloproliferative disease or leukemias. Myd118 gene expression may be present at low levels, entirely absent or altered in such disease states.

Furthermore, the nucleic acid sequences disclosed herein may be used in the detection of aberrations, such as mutations and deletions, in the gene encoding MYD118. For example, the nucleotide sequences disclosed herein may be used to identify and isolate a genomic sequence for myd118. PCR primers can be designed from various portions of the introns and exons of the genomic myd118 that will allow detection of aberrations in the genomic sequence.

Additionally, human MYD118 and the nucleic acid sequences that encode it will provide the basis for pharmaceutical compositions for the treatment of myeloproliferative diseases, such as ALL, AML, CLL, hairy cell leukemia, CML, PV, AMM/MF and ET. For example, nucleic acid sequences that encode MYD118 can be administered to individuals subject to CML or AMM to induce terminal differentiation of granulocytes, thereby arresting cell proliferation.

Administration of MYD118 or nucleic acid sequences that encode it may alleviate the symptoms associated with myeloproliferative diseases and leukemias, such as anemia, fatigue, splenomegaly, hypermetabolism and thrombohemorrhagic complications.

Alternatively, myd118 nucleic acid antisense molecules or antagonists of MYD118 protein may be used to block the activity of human MYD118 in conditions where it would be preferably to block positive regulators of cell growth arrest. For example, MYD118 may be used alone or in combination with other agents in the ex vivo culturing of hematopoietic stem cells intended for autologous transplant to HIV infected individuals or individuals who have undergone chemotherapy or radiation therapy.

10 The present invention also relates, in part, to expression vectors and host cells comprising polynucleotide sequences encoding MYD118 for the in vivo or in vitro production of MYD118 protein.

"Nucleic acid sequence" as used herein refers to an oligonucleotide, nucleotide or polynucleotide sequence, and fragments or portions thereof, and to DNA or RNA of genomic or synthetic origin which may be double-stranded or single-stranded whether representing the sense or antisense strand. As used herein "amino acid sequence" refers to peptide or protein sequences or portions thereof. As used herein, lower case myd118 refers to a nucleic acid sequence whereas upper case MYD118 refers to a protein sequence. As used herein, peptide nucleic acid (PNA) refers to a class of informational molecules that have a neutral "peptide like" backbone combined with nucleotides that allow hybridization to complementary DNA or RNA with higher affinity and specificity than corresponding oligonucleotides (PerSeptive Biosystems, Marlborough MA).

As used herein, MYD118 refers to MYD118 from bovine, ovine, porcine, equine and preferably human, in naturally occurring or in variant form, or from any source, whether natural, synthetic, semi-synthetic or recombinant.

25 As used herein, "naturally occurring" refers to an MYD118 with an amino acid sequence found in nature, and "biologically active" refers to an MYD118 having structural, regulatory or biochemical functions of the naturally occurring MYD118. Likewise, "immunological activity" is defined as the capability of the natural, recombinant or synthetic MYD118 or any oligopeptide thereof, to induce a specific immune response in appropriate animals or cells and to bind with specific antibodies.

The term "derivative" as used herein refers to the chemical modification of MYD118. Illustrative of such modifications would be replacement of hydrogen by an alkyl, acyl, or amino

group. An MYD118 polypeptide derivative would encode a polypeptide which retains essential biological characteristics of MYD118.

As used herein, the term "purified" refers to molecules, either nucleic or amino acid sequences, that are removed from their natural environment and isolated or separated from at least one other component with which they are naturally associated.

The myd118 Coding Sequences

The nucleotide sequence of myd118 (SEQ ID NO:1) is shown in Figure 1. The entire coding region for human myd118 was initially identified within a cDNA library made from human fetal liver/spleen tissue where it was found 1 time in 2899 usable sequences. A BLAST search (Basic Local Alignment Search Tool; Altschul SF (1993) J. Mol. Evol. 36: 290-300; Altschul SF et al (1990) J. Mol. Biol. 215:403-410) comparing the cDNAs of the human fetal liver/spleen library (SPLNFET01) against the primate database of GenBank 91 identified Incyte Clone 25214 as a non-exact match to murine mRNA for myd118 and related family member human gadd45 (NCBI GI number 53291), see Figure 2. The nucleotide sequence for myd118 was identified within Incyte clone 25214 through a computer generated search for nucleotide sequence alignments. The clone was resequenced, and the coding region determined.

Polynucleotide sequences encoding MYD118 were subsequently found in a cDNA library made from non-adherent peripheral blood mononuclear cells (PBMN) where it was found 1 time in 3941 usable sequences. As used herein term "usable sequences" refers to the total number of clones in a library after the removal of vector, nucleotide repeats, contamination, and mitochondrial DNA.

Various portions of the nucleotide sequence encoding MYD118 have been found in GenBank from the following human tissue sources: hippocampus, retina, olfactory epithelium, fetal lung, breast, placenta, white blood cells, brain, pancreas, liver, prostate gland and lung. Polynucleotide sequences encoding MYD118 have not been detected however, in cDNA libraries made from malignant cells of hematopoietic lineage, including THP-1 cells; U937 cells; T/B lymphoblasts from a leukemia source; peripheral blood white blood cells from an individual with myelogenous leukemia; and human mast cells from an individual with mast cell leukemia. The myd118 nucleotide sequence encodes an acidic protein of 160 amino acids having a predicted isoelectric point of 4.4 and discrete hydrophobic and hydrophilic regions as shown in Figure 3.

Methods for DNA sequencing are well known in the art and employ such enzymes as the Klenow fragment of DNA polymerase I, Sequenase® (US Biochemical Corp, Cleveland OH)),

Taq polymerase (Perkin Elmer, Norwalk CT), thermostable T7 polymerase (Amersham, Chicago IL), or combinations of recombinant polymerases and proofreading exonucleases such as the ELONGASE Amplification System marketed by Gibco BRL (Gaithersburg MD). Methods to extend the DNA from an oligonucleotide primer annealed to the DNA template of interest have
5 been developed for both single-stranded and double-stranded templates. Chain termination reaction products were separated using electrophoresis and detected via their incorporated, labeled precursors. Recent improvements in mechanized reaction preparation, sequencing and analysis have permitted expansion in the number of sequences that can be determined per day. Preferably, the process is automated with machines such as the Hamilton Micro Lab 2200
10 (Hamilton, Reno NV), Peltier Thermal Cycler (PTC200; MJ Research, Watertown MA) and the ABI Catalyst 800 and 377 and 373 DNA sequencers (Perkin Elmer).

The quality of any particular cDNA library from which polynucleotides encoding MYD118 are found may be determined by performing a pilot scale analysis of the cDNAs and checking for percentages of clones containing vector, lambda or *E. coli* DNA, mitochondrial or
15 repetitive DNA, and clones with exact or homologous matches to public databases.

Extending myd118 Polynucleotide Sequence

The polynucleotide sequence of myd118 may be extended utilizing the nucleotide sequences from SEQ ID NO:1 in various methods known in the art to detect upstream sequences such as promoters and regulatory elements. Gobinda et al (1993; PCR Methods Applic 2:318-
20 22) disclose "restriction-site polymerase chain reaction (PCR)" as a direct method which uses universal primers to retrieve unknown sequence adjacent to a known locus. First, genomic DNA is amplified in the presence of primer to a linker sequence and a primer specific to the known region. The amplified sequences are subjected to a second round of PCR with the same linker primer and another specific primer internal to the first one. Products of each round of PCR are
25 transcribed with an appropriate RNA polymerase and sequenced using reverse transcriptase.

Inverse PCR can be used to amplify or extend sequences using divergent primers based on a known region (Triglia T et al(1988) Nucleic Acids Res 16:8186). The primers may be designed using Oligo 4.0 (National Biosciences Inc, Plymouth MN), or another appropriate program, to be 22-30 nucleotides in length, to have a GC content of 50% or more, and to anneal
30 to the target sequence at temperatures about 68°-72° C. The method uses several restriction enzymes to generate a suitable fragment in the known region of a gene. The fragment is then circularized by intramolecular ligation and used as a PCR template.

Capture PCR (Lagerstrom M et al (1991) PCR Methods Applic 1:111-19) is a method for PCR amplification of DNA fragments adjacent to a known sequence in human and yeast artificial chromosome (YAC) DNA. Capture PCR also requires multiple restriction enzyme digestions and ligations to place an engineered double-stranded sequence into an unknown portion of the DNA molecule before PCR.

Parker JD et al (1991; Nucleic Acids Res 19:3055-60), teach walking PCR, a method for targeted gene walking which permits retrieval of unknown sequence. PromoterFinder™ is a new kit available from Clontech (Palo Alto CA) which uses PCR, nested primers and special libraries to "walk in" genomic DNA. This process avoids the need to screen libraries and is useful in finding intron/exon junctions.

Another PCR method, "Improved Method for Obtaining Full Length cDNA Sequences" by Guegler et al, Patent Application Serial No 08/487,112, filed June 7, 1995 and hereby incorporated by reference, employs XL-PCR™ enzymes (Perkin-Elmer, Foster City CA) to amplify and/or extend nucleotide sequences.

Preferred libraries for screening for full length cDNAs are ones that have been size-selected to include larger cDNAs. Also, random primed libraries are preferred in that they will contain more sequences which contain the 5' and upstream regions of genes. A randomly primed library may be particularly useful if an oligo d(T) library does not yield a full-length cDNA. Genomic libraries are useful for obtaining introns and extending 5' sequence.

A new method for analyzing either the size or confirming the nucleotide sequence of sequencing or PCR products is capillary electrophoresis. Systems for rapid sequencing are available from Perkin Elmer, Beckman Instruments (Fullerton CA), and other companies. Capillary sequencing employs flowable polymers for electrophoretic separation, four different fluorescent dyes (one for each nucleotide) which are laser activated, and detection of the emitted wavelengths by a charge coupled device camera. Output/light intensity is converted to electrical signal using appropriate software (eg. Genotyper™ and Sequence Navigator™ from Perkin Elmer) and the entire process from loading of samples to computer analysis and electronic data display is computer controlled. Capillary electrophoresis is particularly suited to the sequencing of small pieces of DNA which might be present in limited amounts in a particular sample. The reproducible sequencing of up to 350 bp of M13 phage DNA in 30 min has been reported (Ruiz-Martinez MC et al (1993) Anal Chem 65:2851-8).

Expression of MYD118

In accordance with the present invention, myd118 polynucleotide sequences which encode MYD118 polypeptide sequences, fragments, fusion proteins or functional equivalents thereof, may be used to generate recombinant DNA molecules that direct the expression of myd118 in appropriate host cells. Due to the inherent degeneracy of the genetic code, DNA sequences other than the polynucleotide sequences of SEQ ID NO:1 which encode substantially the same or a functionally equivalent amino acid sequence, may be used to clone and express myd118. As will be understood by those of skill in the art, it may be advantageous to produce MYD118-encoding nucleotide sequences possessing non-naturally occurring codons. Codons preferred by a particular prokaryotic or eukaryotic host (Murray E et al (1989) Nuc Acids Res 17:) can be selected, for example, to increase the rate of myd118 expression or to produce recombinant RNA transcripts having desirable properties, such as a longer half-life, than transcripts produced from naturally occurring sequence.

Also included within the scope of the present invention are polynucleotide sequences that are capable of hybridizing to SEQ ID NO:1 under conditions of intermediate to maximal stringency as long as the polynucleotide sequence capable of hybridizing encodes a protein that retains a biological activity of the naturally occurring MYD118. Hybridization conditions are based on the melting temperature (T_m) of the nucleic acid binding complex, as taught in Wahl GM et al. (1987, Methods Enzymol 152:399-407) incorporated herein by reference, and confer a defined "stringency" as explained below.

"Maximum stringency" typically occurs at about $T_m - 5^\circ\text{C}$ (5°C below the T_m of the probe); "high stringency" at about 5°C to 10°C below T_m ; "intermediate stringency" at about 10°C to 20°C below T_m ; and "low stringency" at about 20°C to 25°C below T_m . As will be understood by those of skill in the art, a maximum stringency hybridization can be used to identify or detect identical polynucleotide sequences while an intermediate (or low) stringency hybridization can be used to identify or detect similar or related polynucleotide sequences.

The term "hybridization" as used herein refers to "the process by which a strand of nucleic acid joins with a complementary strand through base pairing" (Coombs J (1994) Dictionary of Biotechnology, Stockton Press, New York NY). Amplification as carried out in polymerase chain reaction technologies is described in Dieffenbach CW and GS Dveksler (1995, PCR Primer, a Laboratory Manual, Cold Spring Harbor Press, Plainview NY) and incorporated herein by reference.

As used herein a "deletion" is defined as a change in either nucleotide or amino acid

sequence in which one or more nucleotides or amino acid residues, respectively, are absent.

As used herein an "insertion" or "addition" is that change in a nucleotide or amino acid sequence which has resulted in the addition of one or more nucleotides or amino acid residues, respectively, as compared to the naturally occurring molecule.

5 As used herein "substitution" results from the replacement of one or more nucleotides or amino acids by different nucleotides or amino acids, respectively.

Variant myd118 polynucleotide sequences may be used in accordance with the invention and include deletions, insertions or substitutions of different nucleotide residues resulting in a polynucleotide that encodes the same or a functionally equivalent MYD118 polypeptide.

10 Variant MYD118 protein may also be used in accordance with the invention and may include deletions, insertions or substitutions of amino acid residues as long as the result is a functionally equivalent MYD118. As used herein, the term functionally equivalent refers to a variant polynucleotide or variant polypeptide sequence that retains at least one of the biological activities of the naturally occurring sequence. Amino acid substitutions may be made on the basis of
15 similarity in polarity, charge, solubility, hydrophobicity, hydrophilicity, and/or the amphipathic nature of the residues as long as a biological activity of MYD118 is retained. For example, negatively charged amino acids include aspartic acid and glutamic acid; positively charged amino acids include lysine and arginine; and amino acids with uncharged polar head groups having similar hydrophilicity values are grouped as follows: leucine, isoleucine and valine; glycine and
20 alanine; asparagine and glutamine; and lastly, serine, threonine phenylalanine, and tyrosine.

Included within the scope of the present invention are alleles of myd118. As used herein, an "allele" or "allelic sequence" is an alternative form of myd118. Alleles result from a mutation, ie, a change in the nucleic acid sequence, and generally produce altered mRNAs or polypeptides whose structure or function may or may not be altered. Any given gene may have
25 none, one or many allelic forms. Common mutational changes which give rise to alleles are generally ascribed to deletions, additions or substitutions of nucleic acids. Each of these types of changes may occur alone, or in combination with the others, and at the rate of one or more times in a given sequence.

The nucleotide sequences of the present invention may be engineered in order to alter an
30 myd118 coding sequence for a variety of reasons, including but not limited to, alterations which modify the cloning, processing and/or expression of the gene product. For example, mutations may be introduced using techniques which are well known in the art, eg, site-directed

mutagenesis to insert new restriction sites, to alter glycosylation patterns, to change codon preference, etc.

In another embodiment of the invention, an myd118 natural, modified or recombinant sequence may be ligated to a heterologous sequence to encode a fusion protein. For example, for screening of peptide libraries for inhibitors of MYD118 activity, it may be useful to encode a chimeric MYD118 protein expressing a heterologous epitope that is recognized by a commercially available antibody. A fusion protein may also be engineered to contain a cleavage site located between an MYD118 sequence and the heterologous protein sequence, so that the MYD118 may be cleaved and purified away from the heterologous moiety.

In an alternate embodiment of the invention, the coding sequence of myd118 could be synthesized, whole or in part, using chemical methods well known in the art (See Caruthers MH et al (1980) Nuc Acids Res Symp Ser 215-23, Horn T et al(1980) Nuc Acids Res Symp Ser 225-32, etc). Alternatively, the protein itself could be produced using chemical methods to synthesize an MYD118 amino acid sequence, whole or in part. For example, peptides can be synthesized by solid phase techniques, cleaved from the resin, and purified by preparative high performance liquid chromatography (eg, Creighton (1983) Proteins Structures And Molecular Principles, WH Freeman and Co, New York NY). The composition of the synthetic peptides may be confirmed by amino acid analysis or sequencing (eg, the Edman degradation procedure; Creighton, supra)

Direct peptide synthesis can be performed using various solid-phase techniques (Roberge JY et al (1995) Science 269:202-204) and automated synthesis may be achieved, for example, using ABI 431A Peptide Synthesizer (Perkin Elmer) in accordance with the instructions provided by the manufacturer. Additionally the amino acid sequence of MYD118, or any part thereof, may be altered during direct synthesis and/or combined using chemical methods with other sequences to produce a variant polypeptide.

25 Expression Systems

In order to express a biologically active MYD118, the nucleotide sequence coding for MYD118, or a functional equivalent thereof, is inserted into an appropriate expression vector, ie, a vector which contains the necessary elements for the transcription and translation of the inserted coding sequence. Methods which are well known to those skilled in the art can be used to construct expression vectors containing an MYD118 coding sequence and appropriate transcriptional or translational controls. These methods include in vitro recombinant DNA techniques, synthetic techniques and in vivo recombination or genetic recombination. Such

techniques are described in Maniatis et al (1989) Molecular Cloning. A Laboratory Manual, Cold Spring Harbor Press, Plainview NY and Ausubel FM et al. (1989) Current Protocols in Molecular Biology, John Wiley & Sons, New York NY.

Analysis of the functions of growth inhibitory genes, such as *myd118*, by using
5 expression vectors can be technically difficult, since even low-level expression may block growth of the host cell. Stable transfectants that do grow out are often revertants that have lost expression or have had other compensatory changes (Zhan et al. supra). Therefore, a preferred expression system for the expression of MYD118 in host cells is one that allows for expression of proteins toxic to the host cell. For expression in mammalian cells, Zhan et al. (supra, p. 2366),
10 describe a mammalian expression system for the expression of MYD118 related family members GADD45, which has been shown to be regulated by p53, and murine MYD118. This expression system employs the cytomegalovirus promoter and cotransfection with a selectable marker, pSV2neo. Expression studies were performed in human tumor lines with a null p53 phenotype (H1299), in cells with a normal p53 phenotype (RKO), and in cells containing a viral protein, E6,
15 that interferes with p53 function (HeLa).

For expression of MYD118 in *E. coli*, Brown et al. (Gene 1993, 127:99-103) describe a method that was used for expression of the toxic POL3 gene of *S. cerevisiae* which involves the use of the vector pT7SC. Brown obtained up to 15 mg of protein from as little as 3 grams of cells and the expressed protein was in the form of inclusion bodies.

20 Identification of Transformants Containing *myd118*

Although the presence/absence of marker gene expression suggests that the gene of interest is also present, its presence and expression should be confirmed. For example, if the *myd118* is inserted within a marker gene sequence, recombinant cells containing *myd118* can be identified by the absence of marker gene function. Alternatively, a marker gene can be placed in
25 tandem with an *myd118* sequence under the control of a single promoter. Expression of the marker gene in response to induction or selection usually indicates expression of *myd118* as well.

Alternatively, host cells which contain the coding sequence for *myd118* and express *myd118* may be identified by a variety of procedures known to those of skill in the art. These
30 procedures include, but are not limited to, DNA-DNA or DNA-RNA hybridization and protein bioassay or immunoassay techniques which include membrane-based, solution-based, or chip-based technologies for the detection and/or quantification of the nucleic acid or protein.

The presence of the myd118 polynucleotide sequence can be detected by DNA-DNA or DNA-RNA hybridization or amplification using probes, portions or fragments of myd118 disclosed in SEQ ID NO:1. Nucleic acid amplification based assays involve the use of oligonucleotides or oligomers based on the myd118 sequence to detect transformants containing myd118 DNA or RNA. As used herein "oligonucleotides" or "oligomers" refer to a nucleic acid sequence of at least about 10 nucleotides and as many as about 60 nucleotides, preferably about 15 to 30 nucleotides, and more preferably about 20-25 nucleotides which can be used as a probe or amplimer. Preferably, oligonucleotides are derived from the 3' region of the myd118 nucleotide sequence shown in Figure 1.

10 A variety of protocols for detecting and measuring the expression of MYD118 polypeptide, using either polyclonal or monoclonal antibodies specific for the protein are known in the art. Examples include enzyme-linked immunosorbent assay (ELISA), radioimmunoassay (RIA) and fluorescent activated cell sorting (FACS). A two-site, monoclonal-based immunoassay utilizing monoclonal antibodies reactive to two non-interfering epitopes on MYD118 polypeptides is preferred, but a competitive binding assay may be employed. These and other assays are described, among other places, in Hampton R et al (1990, Serological Methods, a Laboratory Manual, APS Press, St Paul MN) and Maddox DE et al (1983, J Exp Med 158:1211).

A wide variety of labels and conjugation techniques are known by those skilled in the art and can be used in various nucleic and amino acid assays. Means for producing labeled hybridization or PCR probes for detecting myd118 polynucleotide sequences include oligolabeling, nick translation, end-labeling or PCR amplification using a labeled nucleotide. Alternatively, the myd118 sequence, or any portion of it, may be cloned into a vector for the production of an mRNA probe. Such vectors are known in the art, are commercially available, and may be used to synthesize RNA probes in vitro by addition of an appropriate RNA polymerase such as T7, T3 or SP6 and labeled nucleotides.

A number of companies such as Pharmacia Biotech (Piscataway NJ), Promega (Madison WI), and US Biochemical Corp (Cleveland OH) supply commercial kits and protocols for these procedures. Suitable reporter molecules or labels include those radionuclides, enzymes, fluorescent, chemiluminescent, or chromogenic agents as well as substrates, cofactors, inhibitors, magnetic particles and the like. Patents teaching the use of such labels include US Patents 3,817,837; 3,850,752; 3,939,350; 3,996,345; 4,277,437; 4,275,149 and 4,366,241. Also,

recombinant immunoglobulins may be produced as shown in US Patent No. 4,816,567 and incorporated herein by reference.

Purification of MYD118

Host cells transformed with a myd118 nucleotide sequence may be cultured under
5 conditions suitable for the expression and recovery of the encoded protein from cell culture. The protein produced by a recombinant cell may be secreted or may be contained intracellularly depending on the sequence and/or the vector used. As will be understood by those of skill in the art, expression vectors containing myd118 can be designed with signal sequences which direct secretion of MYD118 through a particular prokaryotic or eukaryotic cell membrane. Other
10 recombinant constructions may join myd118 to nucleotide sequence encoding a polypeptide domain which will facilitate purification of soluble proteins (Kroll DJ et al (1993) DNA Cell Biol 12:441-53; see also above discussion of vectors containing fusion proteins).

MYD118 may also be expressed as a recombinant protein with one or more additional polypeptide domains added to facilitate protein purification. Such purification facilitating
15 domains include, but are not limited to, metal chelating peptides such as histidine-tryptophan modules that allow purification on immobilized metals (Porath J (1992) Protein Expr Purif 3:263-281), protein A domains that allow purification on immobilized immunoglobulin, and the domain utilized in the FLAGS extension/affinity purification system (Immunex Corp, Seattle WA). The inclusion of a cleavable linker sequences such as Factor XA or enterokinase (Invitrogen, San
20 Diego CA) between the purification domain and MYD118 is useful to facilitate purification.

Uses of MYD118

MYD118 appears to induce terminal differentiation of hematopoietic cells thereby arresting cell proliferation and inducing apoptosis. Polynucleotide sequences encoding MYD118, or portions thereof, have been found in a variety of cDNA libraries from tissues
25 including fetal liver/spleen, PBMN cells, hippocampus, retina, olfactory epithelium, human fetal lung, breast, placenta, human white blood cells, human brain, human pancreas, liver, prostate gland and lung. Polynucleotide sequences encoding MYD118 are not detected in cDNA libraries made from malignant cells of hematopoietic lineage, where genes related to cell growth arrest and apoptosis would be expected to be expressed at undetectable levels or not expressed at all.
30 Based upon 1) its characterization as an MYD family member, 2) the ability of MYD family members to terminally differentiate cells of a hematopoietic lineage and 3) the ability of related MYD family members to reverse a leukemic phenotype *in vivo*, human MYD118 disclosed

herein may be used as a diagnostic or therapeutic agent in the detection or treatment of proliferative diseases of hematopoietic cells, specifically in myeloproliferative diseases and leukemias.

Accordingly, MYD118 can be used to treat or ameliorate the symptoms of
5 myeloproliferative diseases or leukemias, such as acute lymphocytic leukemia (ALL), acute myelogenous leukemia (AML), chronic lymphocytic leukemia (CLL), hairy cell leukemia, chronic myelogenous leukemia (CML); polycythemia vera (PV); agnogenic myeloid metaplasia with myelofibrosis (AMM/MF); and essential thrombocytosis (ET). MYD118 may be used alone or in combination with other drugs or agents in the treatment of such diseases. For example,
10 administration of MYD118 to individuals subject to myeloproliferative disease, which is characterized by the production of increased numbers of immature cells in the peripheral blood, may induce terminal differentiation of the immature cells thereby leading to the maturation of the cells and eventual apoptosis. Administration of MYD118 may be used to augment chemotherapy, such as administration of busulfan, radiation therapy and/or bone marrow
15 transplant. MYD118 may be used therapeutically to ameliorate the symptoms associated with myeloproliferative diseases and leukemias, such as, anemia, fatigue, splenomegaly hypermetabolism and thrombohemorrhagic complications.

MYD118 may also be used to treat disease states related to abnormal proliferation of cells associated with severe inflammation including, but not limited, to rheumatoid arthritis, psoriasis,
20 and lymphomatoid granulomatosis.

In another embodiment of the present invention, antibodies or antagonists of MYD118 may be used in the ex vivo culturing of cells from a hematopoietic cell lineage intended for autologous transplant where it would be desirable to eliminate a cell growth arrest factor that might interfere with desirable cell proliferation.

25 MYD118 Antibodies

Procedures well known in the art may be used for the production of antibodies to MYD118 polypeptides. Such antibodies include, but are not limited to, polyclonal, monoclonal, chimeric, single chain, Fab fragments and fragments produced by a Fab expression library. Neutralizing antibodies, ie, those which inhibit biological activity of MYD118 polypeptides, are
30 especially preferred for diagnostics and therapeutics.

For the production of antibodies, various hosts including goats, rabbits, rats, mice, etc may be immunized by injection with MYD118 polypeptide or any portion, fragment or

oligopeptide which retains immunogenic properties. Depending on the host species, various adjuvants may be used to increase immunological response. Such adjuvants include, but are not limited to, Freund's, mineral gels such as aluminum hydroxide, and surface active substances such as lysolecithin, pluronic polyols, polyanions, peptides, oil emulsions, keyhole limpet hemocyanin, and dinitrophenol. BCG (bacilli Calmette-Guerin) and Corynebacterium parvum are potentially useful human adjuvants which may be employed if purified MYD118 polypeptide is administered to immunologically compromised individuals for the purpose of stimulating systemic defense.

Monoclonal antibodies to MYD118 polypeptide may be prepared using any technique which provides for the production of antibody molecules by continuous cell lines in culture. These include but are not limited to the hybridoma technique originally described by Koehler and Milstein (1975 Nature 256:495-497), the human B-cell hybridoma technique (Kosbor et al (1983) Immunol Today 4:72; Cote et al (1983) Proc Natl Acad Sci 80:2026-2030) and the EBV-hybridoma technique (Cole et al (1985) Monoclonal Antibodies and Cancer Therapy, Alan R Liss Inc, pp 77-96). In addition, techniques developed for the production of "chimeric antibodies", the splicing of mouse antibody genes to human antibody genes to obtain a molecule with appropriate antigen specificity and biological activity can be used (Morrison et al (1984) Proc Natl Acad Sci 81:6851-6855; Neuberger et al (1984) Nature 312:604-608; Takeda et al (1985) Nature 314:452-454). Alternatively, techniques described for the production of single chain antibodies (US Patent No. 4,946,778) can be adapted to produce MYD118 specific single chain antibodies.

Antibodies may also be produced by inducing in vivo production in the lymphocyte population or by screening recombinant immunoglobulin libraries or panels of highly specific binding reagents as disclosed in Orlandi R et al (1989, Proc Natl Acad Sci 86: 3833-3837), and Winter G and Milstein C (1991; Nature 349:293-299).

Antibody fragments which contain specific binding sites for MYD118 may also be generated. For example, such fragments include, but are not limited to, the F(ab')₂ fragments which can be produced by pepsin digestion of the antibody molecule and the Fab fragments which can be generated by reducing the disulfide bridges of the F(ab')₂ fragments. Alternatively, Fab expression libraries may be constructed to allow rapid and easy identification of monoclonal Fab fragments with the desired specificity (Huse WD et al (1989) Science 256:1275-1281).

MYD118-specific antibodies are useful for the diagnosis of conditions and diseases associated with expression of MYD118 polypeptide. A variety of protocols for competitive

binding or immunoradiometric assays using either polyclonal or monoclonal antibodies with established specificities are well known in the art. Such immunoassays typically involve the formation of complexes between MYD118 polypeptides and its specific antibody (or similar MYD118-binding molecule) and the measurement of complex formation. A two-site,
5 monoclonal-based immunoassay utilizing monoclonal antibodies reactive to two noninterfering epitopes on a specific MYD118 protein is preferred, but a competitive binding assay may also be employed. These assays are described in Maddox (supra).

Diagnostic Assays Using MYD118 Specific Antibodies

Anti-MYD118 antibodies are useful for the diagnosis of myeloproliferative disease or
10 leukemias or other conditions, disorders or diseases characterized by abnormal expression of MYD118. Diagnostic assays for MYD118 include methods utilizing the antibody and a label to detect MYD118 polypeptide in human body fluids, cells, tissues or sections or extracts of such tissues. The polypeptides and antibodies of the present invention may be used with or without modification. Frequently, the polypeptides and antibodies will be labeled by joining them, either
15 covalently or noncovalently, with a reporter molecule. A wide variety of reporter molecules are known to those of skill in the art.

A variety of protocols for measuring MYD118 polypeptide, using either polyclonal or monoclonal antibodies specific for the respective protein are known in the art. Examples include enzyme-linked immunosorbent assay (ELISA), radioimmunoassay (RIA) and fluorescent
20 activated cell sorting (FACS). A two-site, monoclonal-based immunoassay utilizing monoclonal antibodies reactive to two non-interfering epitopes on MYD118 polypeptide is preferred, but a competitive binding assay may be employed. These assays are described, among other places, in Maddox (supra).

In order to provide a basis for the diagnosis of disease, normal or standard values for
25 MYD118 polypeptide expression must be established. This is accomplished by combining body fluids or cell extracts taken from normal subjects, either animal or human, with antibody to MYD118 polypeptide under conditions suitable for complex formation which are well known in the art. The amount of standard complex formation may be quantified by comparing it with a dilution series of positive controls where a known amount of antibody is combined with known
30 concentrations of purified MYD118 polypeptide. Then, standard values obtained from normal samples may be compared with values obtained from samples from subjects potentially affected by a disorder or disease related to MYD118 polypeptide expression. Deviation between standard

and subject values establishes the presence of the disease state.

Drug Screening

MYD118 polypeptide, its immunogenic fragments or oligopeptides can be used for screening therapeutic compounds in any of a variety of drug screening techniques. The fragment
5 employed in such a test may be free in solution, affixed to a solid support, borne on a cell surface, or located intracellularly. The abolition of activity or the formation of binding complexes, between MYD118 polypeptide and the agent being tested, may be measured. Accordingly, the present invention provides a method for screening a plurality of compounds for specific binding affinity with MYD118, or a portion thereof, comprising providing a plurality of compounds;
10 combining MYD118, or a portion thereof, with each of a plurality of compounds for a time sufficient to allow binding under suitable conditions; and detecting binding of MYD118, or portion thereof, to each of the plurality of compounds, thereby identifying the compounds which specifically bind MYD118.

Another technique for drug screening provides for high throughput screening of
15 compounds having suitable binding affinity to the MYD118 polypeptides and is described in detail in Geysen, European Patent Application 84/03564, published on September 13, 1984, incorporated herein by reference. In summary, large numbers of different small peptide test compounds are synthesized on a solid substrate, such as plastic pins or some other surface. The peptide test compounds are reacted with MYD118 fragments and washed. Bound MYD118 is
20 then detected by methods well known in the art. Purified MYD118 can also be coated directly onto plates for use in the aforementioned drug screening techniques. Alternatively, non-neutralizing antibodies can be used to capture the peptide and immobilize it on a solid support.

This invention also contemplates the use of competitive drug screening assays in which neutralizing antibodies capable of binding MYD118 specifically compete with a test compound
25 for binding MYD118. In this manner, the antibodies can be used to detect the presence of any peptide which shares one or more antigenic determinants with MYD118.

Uses of myd118 Polynucleotide

An myd118 polynucleotide, or any part thereof, may be used for diagnostic and/or therapeutic purposes. For diagnostic purposes, myd118 polynucleotide sequences may be used to
30 detect and quantitate gene expression in conditions, disorders or diseases in which myd118 activity may be implicated, for example in myeloproliferative diseases or leukemias, where myd118 gene expression appears to be absent, aberrant or not detected. For therapeutic purposes,

myd118 polynucleotide sequences may be administered to individuals with myeloid proliferative disease or leukemia to induce terminal differentiation of proliferating immature hematopoietic cells origin, thereby arresting cell proliferation, ameliorating the symptoms of disease, and reversing the leukemic phenotype.

5 Included in the scope of the invention are oligonucleotide sequences, antisense RNA and DNA molecules and ribozymes, which function to inhibit translation of an myd118. Such nucleotide sequences may be used in conditions where it would be preferable to block positive regulators of cell growth arrest, for example, in ex vivo culturing of cells intended for autologous transplant to individuals lacking cells of the hematopoietic lineage, such as individuals who are
10 immunocompromised due to disease, such as HIV infection, or individuals subject to chemotherapy or radiation therapy which depletes certain cells of hematopoietic lineage.

Another aspect of the subject invention is to provide for nucleic acid hybridization or PCR probes which are capable of detecting polynucleotide sequences, including genomic sequences, encoding MYD118 or closely related molecules, such as alleles. The specificity of
15 the probe, ie, whether it is derived from a highly conserved, conserved or non-conserved region or domain, and the stringency of the hybridization or amplification (high, intermediate or low) will determine whether the probe identifies only naturally occurring myd118, or related sequences. Probes for the detection of related nucleic acid sequences are selected from conserved or highly conserved nucleotide regions of MYD family members and such probes may
20 be used in a pool of degenerate probes. For the detection of identical nucleic acid sequences, or where maximum specificity is desired, nucleic acid probes are selected from the non-conserved nucleotide regions or unique regions of myd118 polynucleotides. As used herein, the term "non-conserved nucleotide region" refers to a nucleotide region that is unique to myd118 and does not occur in related family members, such as the nucleotide sequence encoding GADD45.

25

Diagnostic Uses of myd118 Polynucleotide

An myd118 encoding polynucleotide sequence may be used for the diagnosis of diseases resulting from abnormal expression of myd118 or other genes associated with myeloproliferative
30 disease or leukemia. For example, polynucleotide sequences encoding MYD118 may be used in hybridization or PCR assays of tissues from biopsies or autopsies or biological fluids, such as serum, to detect abnormalities in myd118 expression. The form of such qualitative or

quantitative methods may include Southern or northern analysis, dot blot or other membrane-based technologies; PCR technologies; dip stick, pin or chip technologies; and ELISA or other multiple sample format technologies. All of these techniques are well known in the art, and are in fact the basis of many commercially available diagnostic kits.

5 Such assays may be tailored to evaluate the efficacy of a particular therapeutic treatment regime and may be used in animal studies, in clinical trials, or in monitoring the treatment of an individual patient. In order to provide a basis for the diagnosis of disease, a normal or standard profile for myd118 expression must be established. This is accomplished by combining body fluids or cell extracts taken from normal subjects, either animal or human, with myd118 or a
10 portion thereof, under conditions suitable for hybridization or amplification. Standard hybridization may be quantified by comparing the values obtained for normal subjects with a dilution series of positive controls run in the same experiment where a known amount of purified myd118 is used. Standard values obtained from normal samples may be compared with values obtained from samples from subjects potentially affected by a disorder or disease related to
15 myd118 expression. Deviation between standard and subject values establishes the presence of the disease state. If disease is established, an existing therapeutic agent is administered, and treatment profile or values may be generated. Finally, the assay may be repeated on a regular basis to evaluate whether the values progress toward or return to the normal or standard pattern. Successive treatment profiles may be used to show the efficacy of treatment over a period of
20 several days or several months.

 PCR as described in US Patent Nos. 4,683,195; 4,800,195; and 4,965,188 provides additional uses for oligonucleotides based upon the myd118 sequence. Such oligomers are generally chemically synthesized, but they may be generated enzymatically or produced from a recombinant source. Oligomers generally comprise two nucleotide sequences, one with sense
25 orientation (5'→3') and one with antisense (3'←5') employed under optimized conditions for identification of a specific gene or condition. The same two oligomers, nested sets of oligomers, or even a degenerate pool of oligomers may be employed under less stringent conditions for detection and/or quantitation of closely related DNA or RNA sequences.

 Additionally methods to quantitate the expression of a particular molecule include
30 radiolabeling (Melby PC et al 1993 J Immunol Methods 159:235-44) or biotinylating (Duplaa C et al 1993 Anal Biochem 229-36) nucleotides, coamplification of a control nucleic acid, and standard curves onto which the experimental results are interpolated. Quantitation of multiple

samples may be speeded up by running the assay in an ELISA format where the an oligomer of interest is presented in various dilutions and a spectrophotometric or colorimetric response gives rapid quantitation.

Therapeutic Uses of an myd118 Polynucleotide

5 An myd118 polynucleotide sequence may provide the basis for treatment of various abnormal conditions involving abnormally proliferating cells of hematopoietic lineage, including myeloproliferative diseases and leukemias, such as acute lymphocytic leukemia (ALL), acute myelogenous leukemia (AML), chronic lymphocytic leukemia (CLL), hairy cell leukemia, chronic myelogenous leukemia (CML); polycythemia vera (PV); agnogenic myeloid metaplasia
10 with myelofibrosis (AMM/MF); and essential thrombocytosis (ET), where it would be advantageous to induce terminal differentiation of the proliferating cells thereby arresting cell proliferation. Stable transformation of appropriate germ line cells, or a zygote, with a vector containing polynucleotide sequences encoding MYD118 may produce a transgenic organism (US Patent No. 4,736,866, 12 April 1988) producing enough copies of the polynucleotide sequence to
15 induce terminal differentiation of cells of hematopoietic lineage, thereby leading to cell growth arrest. Such vectors and expression systems intended for in vivo or gene therapy use should be designed for expression of molecules toxic to the host cell as disclosed infra. A preferable expression vector would be one which drives expression of myd118 at levels comparable to its expression in normal non-proliferating cells.

20 Alternatively, an myd118 polynucleotide sequence may also provide the basis for design of antisense molecules that are able to inhibit transcription or translation of myd118 in conditions where it would be advantageous to block positive regulators of cell growth arrest, such as for example in ex vivo culturing of cells from a hematopoietic lineage intended for autologous transplant, where proliferation of cells of hematopoietic lineage would be desirable. The
25 introduction of vectors into stem cells taken from a patient and clonally propagated for autologous transplant is described in US Patent Nos. 5,399,493 and 5,437,994, disclosed herein by reference.

Expression vectors derived from retroviruses, adenovirus, herpes or vaccinia viruses, or from various bacterial plasmids, may be used for delivery of recombinant myd118, sense or
30 antisense molecules, to the targeted cell population. Methods which are well known to those skilled in the art can be used to construct recombinant vectors containing myd118. See, for example, the techniques described in Maniatis et al (supra) and Ausubel et al(supra).

Alternatively, recombinant myd118 can be delivered to target cells in liposomes.

The full length cDNA sequence and/or its regulatory elements enable researchers to use myd118 as a tool in sense (Youssoufian H and HF Lodish 1993 Mol Cell Biol 13:98-104) or antisense (Eguchi et al (1991) Annu Rev Biochem 60:631-652) investigations of gene function.

5 Oligonucleotides, designed from the cDNA or control sequences obtained from the genomic DNA can be used in vitro or in vivo to inhibit expression. Such technology is now well known in the art, and sense or antisense oligonucleotides or larger fragments can be designed from various locations along the coding or control regions.

Additionally, myd118 expression can be modulated by transfecting a cell or tissue with
10 expression vectors which express high levels of an myd118 fragment in conditions where it would be preferably to block the activity of positive regulator of cell growth arrest, such as in bone marrow transplant therapy and ex vivo culturing of cells intended for autologous transplant. Such constructs can flood cells with untranslatable sense or antisense sequences. Even in the absence of integration into the DNA, such vectors may continue to transcribe RNA molecules
15 until all copies of the vector are disabled by endogenous nucleases. Such transient expression may last for a month or more with a non-replicating vector (Mettler I, personal communication) and even longer if appropriate replication elements are part of the vector system.

Modifications of gene expression can be obtained by designing antisense sequences to the control regions of the myd118 gene, such as the promoters, enhancers, and introns.

20 Oligonucleotides derived from the transcription initiation site, eg, between -10 and +10 regions of the leader sequence, are preferred. Antisense RNA and DNA molecules may also be designed to block translation of mRNA by preventing the transcript from binding to ribosomes. Similarly, inhibition can be achieved using Hogeboom base-pairing methodology, also known as "triple helix" base pairing. Triple helix pairing compromises the ability of the double helix to open
25 sufficiently for the binding of polymerases, transcription factors, or regulatory molecules.

Ribozymes are enzymatic RNA molecules capable of catalyzing the specific cleavage of RNA. The mechanism of ribozyme action involves sequence specific hybridization of the ribozyme molecule to complementary target RNA, followed by an endonucleolytic cleavage. Within the scope of the invention are engineered hammerhead motif ribozyme molecules that
30 specifically and efficiently catalyze endonucleolytic cleavage of myd118 RNA sequences.

Specific ribozyme cleavage sites within any potential RNA target are initially identified by scanning the target molecule for ribozyme cleavage sites which include the following

sequences, GUA, GUU and GUC. Once identified, short RNA sequences of between 15 and 20 ribonucleotides corresponding to the region of the target gene containing the cleavage site may be evaluated for secondary structural features which may render the oligonucleotide sequence inoperable. The suitability of candidate targets may also be evaluated by testing accessibility to hybridization with complementary oligonucleotides using ribonuclease protection assays.

Both antisense RNA and DNA molecules and ribozymes of the invention may be prepared by any method known in the art for the synthesis of RNA molecules. These include techniques for chemically synthesizing oligonucleotides such as solid phase phosphoramidite chemical synthesis. Alternatively, RNA molecules may be generated by in vitro or in vivo transcription of DNA sequences encoding the antisense RNA molecule. Such DNA sequences may be incorporated into a wide variety of vectors with suitable RNA polymerase promoters such as T7 or SP6. Alternatively, antisense cDNA constructs that synthesize antisense RNA constitutively or inducibly can be introduced into cell lines, cells or tissues.

DNA molecules may be modified to increase intracellular stability and half-life. Possible modifications include, but are not limited to, the addition of flanking sequences of the 5' and/or 3' ends of the molecule or the use of phosphorothioate or 2' O-methyl rather than phosphodiesterase linkages within the backbone of the molecule.

Methods for introducing vectors into cells or tissue include those methods discussed infra. In addition, several of these transformation or transfection methods are equally suitable for the ex vivo therapy,

Furthermore, the *myd118* polynucleotide sequences disclosed herein may be used in molecular biology techniques that have not yet been developed, provided the new techniques rely on properties of nucleotide sequences that are currently known, including but not limited to such properties as the triplet genetic code and specific base pair interactions.

25 Detection and Mapping of Polynucleotide Sequences Related to *myd118*

The nucleic acid sequence for *myd118* can also be used to generate hybridization probes as previously described, for mapping the endogenous genomic sequence. The sequence may be mapped to a particular chromosome or to a specific region of the chromosome using well known techniques. These include in situ hybridization to chromosomal spreads (Verma et al (1988)

Human Chromosomes: A Manual of Basic Techniques, Pergamon Press, New York City), flow-sorted chromosomal preparations, or artificial chromosome constructions such as YACs, bacterial artificial chromosomes (BACs), bacterial P1 constructions or single chromosome cDNA libraries.

In situ hybridization of chromosomal preparations and physical mapping techniques such as linkage analysis using established chromosomal markers are invaluable in extending genetic maps. Examples of genetic maps can be found in Science (1995; 270:410f and 1994; 265:1981f). Often the placement of a gene on the chromosome of another mammalian species may reveal associated markers even if the number or arm of a particular human chromosome is not known. New sequences can be assigned to chromosomal arms, or parts thereof, by physical mapping. This provides valuable information to investigators searching for disease genes using positional cloning or other gene discovery techniques. Once a disease or syndrome, such as ataxia telangiectasia (AT), has been crudely localized by genetic linkage to a particular genomic region, for example, AT to 11q22-23 (Gatti et al (1988) Nature 336:577-580), any sequences mapping to that area may represent associated or regulatory genes for further investigation. The nucleotide sequence of the subject invention may also be used to detect differences in the chromosomal location due to translocation, inversion, etc between normal, carrier or affected individuals.

Pharmaceutical Compositions

The present invention relates to pharmaceutical compositions which may comprise all or portions of myd118 polynucleotide sequences, MYD118 polypeptides, inhibitors or antagonists of MYD118 bioactivity, including antibodies, alone or in combination with at least one other agent, such as stabilizing compound, and may be administered in any sterile, biocompatible pharmaceutical carrier, including, but not limited to, saline, buffered saline, dextrose, and water.

Myd118 nucleotide and MYD118 amino acid sequences can be administered to a patient alone, or in combination with other nucleotide sequences, such as nucleotide sequences encoding tumor suppressor genes, such as p53, p16 and p21, drugs or hormones or in pharmaceutical compositions where it is mixed with excipient(s) or other pharmaceutically acceptable carriers. In one embodiment of the present invention, the pharmaceutically acceptable carrier is pharmaceutically inert. A preferred route of administration for treatment of myeloproliferative diseases or leukemias would be intravenous delivery whereas a preferred route of administration for treatment of conditions related to inflammation would be local administration at the site of inflammation, such as the joint affected in rheumatoid arthritis.

Myd118 polynucleotide sequences or MYD118 amino acid sequences may be administered alone to individuals subject to myeloproliferative diseases or leukemias or in combination with other types of agents or therapy including chemotherapy, radiation therapy or stem cell transplant therapy.

Depending on the condition being treated, these pharmaceutical compositions may be formulated and administered systemically or locally. Techniques for formulation and administration may be found in the latest edition of "Remington's Pharmaceutical Sciences" (Mack Publishing Co, Easton PA). Suitable routes may, for example, include oral or
5 transmucosal administration; parenteral delivery, including intramuscular, subcutaneous, intramedullary, intrathecal, intraventricular, intravenous, intraperitoneal, or intranasal administration.

For injection, the pharmaceutical compositions of the invention may be formulated in aqueous solutions, preferably in physiologically compatible buffers such as Hanks's solution,
10 Ringer's solution, or physiologically buffered saline. For tissue or cellular administration, penetrants appropriate to the particular barrier to be permeated are used in the formulation. Such penetrants are generally known in the art.

The pharmaceutical compositions can be formulated using pharmaceutically acceptable carriers well known in the art in dosages suitable for oral administration. Such carriers enable the
15 pharmaceutical compositions to be formulated as tablets, pills, capsules, liquids, gels, syrups, slurries, suspensions and the like, for oral or nasal ingestion by a patient to be treated.

Pharmaceutical compositions suitable for use in the present invention include compositions wherein the active ingredients are contained in an effective amount to achieve the intended purpose. For example, an effective amount of MYD118 may be that amount that
20 ameliorates the symptoms of anemia, fatigue, splenomegaly, hypermetabolism and thrombohemorrhagic complications. Determination of effective amounts is well within the capability of those skilled in the art, especially in light of the disclosure provided below.

In addition to the active ingredients these pharmaceutical compositions may contain suitable pharmaceutically acceptable carriers comprising excipients and auxiliaries which
25 facilitate processing of the active compounds into preparations which can be used pharmaceutically. The preparations formulated for oral administration may be in the form of tablets, dragees, capsules, or solutions.

The pharmaceutical compositions of the present invention may be manufactured in a manner that is itself known, eg, by means of conventional mixing, dissolving, granulating,
30 dragee-making, levigating, emulsifying, encapsulating, entrapping or lyophilizing processes.

Pharmaceutical formulations for parenteral administration include aqueous solutions of the active compounds in water-soluble form. Additionally, suspensions of the active compounds

may be prepared as appropriate oily injection suspensions. Suitable lipophilic solvents or vehicles include fatty oils such as sesame oil, or synthetic fatty acid esters, such as ethyl oleate or triglycerides, or liposomes. Aqueous injection suspensions may contain substances which increase the viscosity of the suspension, such as sodium carboxymethyl cellulose, sorbitol, or 5 dextran. Optionally, the suspension may also contain suitable stabilizers or agents which increase the solubility of the compounds to allow for the preparation of highly concentrated solutions.

Pharmaceutical preparations for oral use can be obtained by combining the active compounds with solid excipient, optionally grinding a resulting mixture, and processing the 10 mixture of granules, after adding suitable auxiliaries, if desired, to obtain tablets or dragee cores. Suitable excipients are carbohydrate or protein fillers such as sugars, including lactose, sucrose, mannitol, or sorbitol; starch from corn, wheat, rice, potato, etc; cellulose such as methyl cellulose, hydroxypropylmethyl-cellulose, or sodium carboxymethylcellulose; and gums including arabic and tragacanth; and proteins such as gelatin and collagen. If desired, 15 disintegrating or solubilizing agents may be added, such as the cross-linked polyvinyl pyrrolidone, agar, alginic acid or a salt thereof such as sodium alginate.

Dragee cores are provided with suitable coatings such as concentrated sugar solutions, which may also contain gum arabic, talc, polyvinylpyrrolidone, carbopol gel, polyethylene glycol, and/or titanium dioxide, lacquer solutions, and suitable organic solvents or solvent 20 mixtures. Dyestuffs or pigments may be added to the tablets or dragee coatings for product identification or to characterize the quantity of active compound, ie, dosage.

Pharmaceutical preparations which can be used orally include push-fit capsules made of gelatin, as well as soft, sealed capsules made of gelatin and a coating such as glycerol or sorbitol. The push-fit capsules can contain the active ingredients mixed with a filler or binders such as 25 lactose or starches, lubricants such as talc or magnesium stearate, and, optionally, stabilizers. In soft capsules, the active compounds may be dissolved or suspended in suitable liquids, such as fatty oils, liquid paraffin, or liquid polyethylene glycol with or without stabilizers.

Compositions comprising a compound of the invention formulated in a pharmaceutical acceptable carrier may be prepared, placed in an appropriate container, and labeled for treatment 30 of an indicated condition. For polynucleotide or amino acid sequences of MYD118, conditions indicated on the label may include treatment of myeloproliferative diseases or leukemias.

The pharmaceutical composition may be provided as a salt and can be formed with many

acids, including but not limited to hydrochloric, sulfuric, acetic, lactic, tartaric, malic, succinic, etc. Salts tend to be more soluble in aqueous or other protonic solvents that are the corresponding free base forms. In other cases, the preferred preparation may be a lyophilized powder in 1 mM-50 mM histidine, 0.1%-2% sucrose, 2%-7% mannitol at a pH range of 4.5 to 5.5 that is combined with buffer prior to use.

For any compound used in the method of the invention, the therapeutically effective dose can be estimated initially from cell culture assays. Then, preferably, dosage can be formulated in animal models (particularly murine models) to achieve a desirable circulating concentration range that adjusts MYD118 levels.

10 A therapeutically effective dose refers to that amount of MYD118 which ameliorates symptoms of the disease state. Toxicity and therapeutic efficacy of such compounds can be determined by standard pharmaceutical procedures in cell cultures or experimental animals, eg, for determining the LD50 (the dose lethal to 50% of the population) and the ED50 (the dose therapeutically effective in 50% of the population). The dose ratio between toxic and therapeutic effects is the therapeutic index, and it can be expressed as the ratio LD50/ED50. Compounds 15 which exhibit large therapeutic indices are preferred. The data obtained from these cell culture assays and additional animal studies can be used in formulating a range of dosage for human use. The dosage of such compounds lies preferably within a range of circulating concentrations that include the ED50 with little or no toxicity. The dosage varies within this range depending upon 20 the dosage form employed, sensitivity of the patient, and the route of administration.

The exact dosage is chosen by the individual physician in view of the patient to be treated. Dosage and administration are adjusted to provide sufficient levels of the active moiety or to maintain the desired effect. Additional factors which may be taken into account include the severity of the disease state; age, weight, and gender of the patient; diet, time and frequency of 25 administration, drug combination(s), reaction sensitivities, and tolerance/response to therapy. Long acting pharmaceutical compositions might be administered every 3 to 4 days, every week, or once every two weeks depending on half-life and clearance rate of the particular formulation.

Normal dosage amounts may vary from 0.1 to 100,000 micrograms, up to a total dose of about 1 g, depending upon the route of administration. Guidance as to particular dosages and 30 methods of delivery is provided in the literature. See US Patent No. 4,657,760; 5,206,344; or 5,225,212. Those skilled in the art will employ different formulations for MYD118 than for the inhibitors of MYD118. Administration to the bone marrow may necessitate delivery in a manner

different from intravenous injections.

These examples are provided by way of illustration and are not included for the purpose of limiting the invention.

5

INDUSTRIAL APPLICABILITY

I SPLNFET01 cDNA Library Construction and Isolation of cDNA Clones

The human spleen cell cDNA library (SPLNFET01) was custom constructed by Stratagene. The tissue was obtained from fetal spleens pooled from different sources and contained many different types of cells. Poly(A⁺) RNA (mRNA) was purified, and cDNA was
10 synthesized from the mRNA. Synthetic adaptor oligonucleotides were ligated onto cDNA ends enabling its insertion into Uni-ZAP™ vector system (Stratagene), allowing high efficiency unidirectional (sense orientation) lambda library construction and the convenience of a plasmid system with blue/white color selection to detect clones with cDNA insertions. Alternative unidirectional vectors are pcDNA1 (Invitrogen, San Diego CA) and pSHlox-1 (Novagen,
15 Madison WI).

The custom-constructed library phage particles were transfected into *E. coli* host strain XL1-Blue® (Stratagene), which has a high transformation efficiency, increasing the probability of obtaining rare, under-represented clones in the cDNA library.

The phagemid forms of individual cDNA clones were obtained by the in vivo excision
20 process, in which the host bacterial strain was coinfectd with both the lambda library phage and an f1 helper phage. Proteins derived from both the library-containing phage and the helper phage nicked the lambda DNA, initiated new DNA synthesis from defined sequences on the lambda target DNA and created a smaller, single stranded circular phagemid DNA molecule that included all DNA sequences of the pBluescript® plasmid and the cDNA insert. The phagemid
25 DNA was secreted from the cells and purified, then used to re-infect fresh host cells, where the double stranded phagemid DNA was produced. Because the phagemid carries the gene for β -lactamase, the newly-transformed bacteria were selected on medium containing ampicillin.

Phagemid DNA was purified using the QIAwell-8 Plasmid, QIAwell PLUS, or QIAwell ULTRA DNA purification system (QIAGEN Inc Chatsworth CA). This product line provides a
30 convenient, rapid and reliable high-throughput method to lyse bacterial cells and isolate highly purified phagemid DNA using QIAGEN anion-exchange resin particles with EMPORE™ membrane technology (3M, Minneapolis MN) in a multiwell format. The DNA was eluted from

the purification resin already prepared for DNA sequencing and other analytical manipulations.

The cDNAs were sequenced by the method of Sanger F and AR Coulson (1975; J Mol Biol 94:441f), using the Catalyst 800 and 373 DNA Sequencing System (Perkin Elmer).

II Homology Searching of cDNA Clones and Their Deduced Proteins

5 Each cDNA was compared to sequences in GenBank using a search algorithm d incorporated into the ABI INHERIT™ 670 Sequence Analysis System (Perkin Elmer). In this algorithm, Pattern Specification Language (TRW Inc, Los Angeles CA) was used to determine regions of homology. The three parameters that determine how the sequence comparisons run were window size, window offset, and error tolerance. Using a combination of these three
10 parameters, the DNA database was searched for sequences containing regions of homology to the query sequence, and the appropriate sequences were scored with an initial value. Subsequently, these homologous regions were examined using dot matrix homology plots to distinguish regions of homology from chance matches. Smith-Waterman alignments were used to display the results of the homology search.

15 BLAST, which stands for Basic Local Alignment Search Tool (Altschul SF (1993) J Mol Evol 36:290-300; Altschul, SF et al (1990) J Mol Biol 215:403-10), was used to search for local sequence alignments. BLAST produces alignments of both nucleotide and amino acid sequences to determine sequence similarity. Because of the local nature of the alignments, BLAST is especially useful in determining exact matches or in identifying homologs. BLAST is useful for
20 matches which do not contain gaps. The fundamental unit of BLAST algorithm output is the High-scoring Segment Pair (HSP).

An HSP consists of two sequence fragments of arbitrary but equal lengths whose alignment is locally maximal and for which the alignment score meets or exceeds a threshold or cutoff score set by the user. The BLAST approach is to look for HSPs between a query sequence
25 and a database sequence, to evaluate the statistical significance of any matches found, and to report only those matches which satisfy the user-selected threshold of significance. The parameter E establishes the statistically significant threshold for reporting database sequence matches. E is interpreted as the upper bound of the expected frequency of chance occurrence of an HSP (or set of HSPs) within the context of the entire database search. Any database sequence
30 whose match satisfies E is reported in the program output.

MYD118 was identified using the ABI INHERIT™ DNA Analysis System (Perkin Elmer) software which identified clone 25214 as being related to human GADD45 and murine

MYD118. PCR extension analysis was performed to determine the 5' coding region. The nucleotide sequence for clone 25214 was subjected to a confirmatory DNA sequence analysis. The polynucleotide (SEQ ID NO:1) and amino acid sequence (SEQ ID NO:2) of MYD118 are disclosed herein in Figure 1.

5 III Determination of Reading Frame of cDNA Clone

The reading frame of individual cDNA clones obtained from the SPLNFET01 library was obtained by analyzing the polynucleotide sequences for the presence of start (ATG, GTG, etc.) and stop codons (TGA, TAA, TAG). Typically, one frame will continue throughout the major portion of all of a cDNA sequence and the other two pending frames tend to contain numerous
10 stop codons. Algorithms for determining reading frame have been developed which analyze the occurrence of individual nucleotide bases of each putative codon triplet (eg, Fickett, JW (1982) Nucleic Acids Res 10:5303-18). Coding DNA tends to contain predominantly certain nucleotides within certain triplet periodicities, such as a significant preference for pyrimidines in the third codon position. These algorithms have been incorporated into widely available software
15 and can be easily used to determine coding potential (and frame) of a given stretch of DNA. This algorithm-derived information, combined with start/stop codon information, was used to determine proper frame of individual clones within the SPLNFET01 library with a high degree of certainty, thus permitting the correct reading frame alignment with appropriate expression vehicles.

20 IV Extension of myd118 to Recover Regulatory Elements

The nucleic acid sequence of myd118 may be used to design oligonucleotide primers for obtaining full length sequences from genomic libraries. One primer is synthesized to initiate extension in the antisense direction (XLR) and the other is synthesized to extend sequence in the sense direction (XLF). The primers allow the known myd118 sequence to be extended
25 "outward" generating amplicons containing new, unknown nucleotide sequence for the control region of interest. The initial primers are designed from the cDNA using Oligo 4.0 (National Biosciences Inc, Plymouth MN), or another appropriate program, to be 22-30 nucleotides in length, to have a GC content of 50% or more, and to anneal to the target sequence at temperatures about 68°-72° C. Any stretch of nucleotides which would result in hairpin structures and
30 primer-primer dimerizations is avoided.

A human genomic library is used to extend and amplify 5' upstream sequence. If necessary, a second set of primers is designed to further extend the known region. By following

the instructions for the XL-PCR kit (Perkin Elmer) and thoroughly mixing the enzyme and reaction mix, high fidelity amplification is obtained. Beginning with 40 pmol of each primer and the recommended concentrations of all other components of the kit, PCR is performed using the Peltier Thermal Cycler (PTC200; MJ Research, Watertown MA) and the following parameters:

- 5 Step 1 94° C for 1 min (initial denaturation)
- Step 2 65° C for 1 min
- Step 3 68° C for 6 min
- Step 4 94° C for 15 sec
- Step 5 65° C for 1 min
- 10 Step 6 68° C for 7 min
- Step 7 Repeat step 4-6 for 15 additional cycles
- Step 8 94° C for 15 sec
- Step 9 65° C for 1 min
- Step 10 68° C for 7:15 min
- 15 Step 11 Repeat step 8-10 for 12 cycles
- Step 12 72° C for 8 min
- Step 13 4° C (and holding)

A 5-10 μ l aliquot of the reaction mixture is analyzed by electrophoresis on a low
 20 concentration (about 0.6-0.8%) agarose mini-gel to determine which reactions were successful in extending the sequence. The largest products or bands were selected and cut out of the gel. Further purification involves using a commercial gel extraction method such as QIAQuick™ (QIAGEN Inc). After recovery of the DNA, Klenow enzyme was used to trim single-stranded, nucleotide overhangs creating blunt ends which facilitate religation and cloning.

25 After ethanol precipitation, the products are redissolved in 13 μ l of ligation buffer, 1 μ l T4-DNA ligase (15 units) and 1 μ l T4 polynucleotide kinase are added, and the mixture is incubated at room temperature for 2-3 hours or overnight at 16° C. Competent *E. coli* cells (in 40 μ l of appropriate media) are transformed with 3 μ l of ligation mixture and cultured in 80 μ l of SOC medium (Sambrook J et al, supra). After incubation for one hour at 37° C, the whole
 30 transformation mixture is plated on Luria Bertani (LB)-agar (Sambrook J et al, supra) containing 2x Carb. The following day, several colonies are randomly picked from each plate and cultured in 150 μ l of liquid LB/2xCarb medium placed in an individual well of an appropriate, commercially-available, sterile 96-well microtiter plate. The following day, 5 μ l of each overnight culture is transferred into a non-sterile 96-well plate and after dilution 1:10 with water,
 35 5 μ l of each sample is transferred into a PCR array.

For PCR amplification, 18 μ l of concentrated PCR reaction mix (3.3x) containing 4 units of rTth DNA polymerase, a vector primer and one or both of the gene specific primers used for

the extension reaction are added to each well. Amplification is performed using the following conditions:

- Step 1 94° C for 60 sec
- Step 2 94° C for 20 sec
- 5 Step 3 55° C for 30 sec
- Step 4 72° C for 90 sec
- Step 5 Repeat steps 2-4 for an additional 29 cycles
- Step 6 72° C for 180 sec
- Step 7 4° C (and holding)

- 10 Aliquots of the PCR reactions are run on agarose gels together with molecular weight markers. The sizes of the PCR products are compared to the original partial cDNAs, and appropriate clones are selected, ligated into plasmid and sequenced.

V Labeling of Hybridization Probes

- Hybridization probes derived from SEQ ID NO:1 may be employed to screen cDNAs,
15 mRNAs or genomic DNAs. Although the labeling of oligonucleotides, consisting of about 20 base-pairs, is specifically described, essentially the same procedure may be used with larger cDNA fragments. Oligonucleotides are labeled by combining 50 pmol of each oligomer and 250 mCi of [g - ^{32}P] adenosine triphosphate (Amersham, Chicago IL) and T4 polynucleotide kinase (DuPont NEN®, Boston MA). The labeled oligonucleotides are purified with Sephadex G-25
20 super fine resin column (Pharmacia). A portion containing 10^7 counts per minute of each is used in a typical membrane based hybridization analysis of human genomic DNA digested with one of the following endonucleases (Ase I, Bgl II, EcoR I, Pst I, Xba I, or Pvu II; DuPont NEN®).

- The DNA from each digest is fractionated on a 0.7 percent agarose gel and transferred to nylon membranes (Nytran Plus, Schleicher & Schuell, Durham NH). Hybridization is carried out
25 for 16 hours at 40°C. To remove nonspecific signals, blots are sequentially washed at room temperature under increasingly stringent conditions up to 0.1 x saline sodium citrate and 0.5% sodium dodecyl sulfate. After XOMAT AR™ film (Kodak, Rochester NY) is exposed to the blots in a Phosphorimager cassette (Molecular Dynamics, Sunnyvale CA) for several hours, hybridization patterns are compared visually.

30 VI Antisense Molecules

- The myd118 sequence, or any part thereof, may be used to inhibit in vivo or in vitro expression of endogenous myd118. Although use of antisense oligonucleotides, consisting of about 20 base-pairs, is specifically described, essentially the same procedure may be used with larger cDNA fragments. An oligonucleotide based on the coding sequence of myd118 may be

used to inhibit expression of endogenous myd118. Using Oligo 4.0, the complementary oligonucleotide can be designed from the conserved 5' sequence and used either to inhibit transcription by preventing promoter binding to the upstream nontranslated sequence or translation of an myd118 transcript by preventing the ribosome from binding to the mRNA.

5 VII Production of MYD118 Specific Antibodies

For production of polyclonal antibodies, the deduced amino acid sequence of MYD118 is analyzed using DNASTAR software (DNASTAR Inc) to determine regions of high immunogenicity and a corresponding oligopeptide is synthesized and used to raise antibodies in rabbits. Analysis to select appropriate epitopes, such as those near the C-terminus or in adjacent
10 hydrophilic regions is described by Ausubel FM et al (supra). An oligopeptide of about 15 residues in length is synthesized using an ABI Peptide Synthesizer Model 431A (Perkin Elmer, Norwalk, CN) using fmoc-chemistry, and coupled to keyhole limpet hemocyanin (KLH, Sigma) by reaction with M-maleimidobenzoyl-N-hydroxysuccinimide ester (MBS; Ausubel FM et al, supra). Rabbits are immunized with the oligopeptide-KLH complex in complete Freund's
15 adjuvant. The resulting antisera are tested for antipeptide activity, for example, by binding the peptide to plastic, blocking with 1% BSA, reacting with rabbit antisera, washing, and reacting with radioiodinated, goat anti-rabbit IgG.

VIII Purification of MYD118 Using Specific Antibodies

Endogenous or recombinant MYD118 can be purified by immunoaffinity
20 chromatography using antibodies specific for MYD118. An immunoaffinity column is constructed by covalently coupling MYD118 antibody to an activated chromatographic resin such as CnBr-activated Sepharose (Pharmacia Biotech). After the coupling, the resin is blocked and washed according to the manufacturer's instructions.

Media containing MYD118 is passed over the immunoaffinity column, and the column is
25 washed under conditions that allow the preferential absorbance of MYD118 (eg, high ionic strength buffers in the presence of detergent). The column is eluted under conditions that disrupt antibody/MYD118 binding (eg, a buffer of pH 2-3 or a high concentration of a chaotrope such as urea or thiocyanate ion), and MYD118 is collected.

IX Identification of Molecules Which Interact with MYD118

30 MYD118, or biologically active fragments thereof, are labeled with ¹²⁵I Bolton-Hunter reagent (Bolton, AE and Hunter, WM (1973) Biochem J 133: 529). Candidate small molecules previously arrayed in the wells of a 96 well plate are incubated with the labeled MYD118,

washed and any wells with labeled MYD118 complex are assayed. Data obtained using different concentrations of MYD118 are used to calculate values for the number, affinity, and association of MYD118 with the candidate molecules.

5 All publications and patents mentioned in the above specification are herein incorporated by reference. Various modifications and variations of the described methods and system of the invention will be apparent to those skilled in the art without departing from the scope and spirit of the invention. Although the invention has been described in connection with specific preferred
10 embodiments, it should be understood that the invention as claimed should not be unduly limited to such specific embodiments. Indeed, various modifications of the described modes for carrying out the invention which are obvious to those skilled in molecular biology or related fields are intended to be within the scope of the following claims.

SEQUENCE LISTING

(1) GENERAL INFORMATION

- (i) APPLICANT: INCYTE PHARMACEUTICALS, INC.
- (ii) TITLE OF THE INVENTION: NOVEL HUMAN MYELOID TERMINAL DIFFERENTIATION RESPONSE GENE
- (iii) NUMBER OF SEQUENCES: 4
- (iv) CORRESPONDENCE ADDRESS:
 - (A) ADDRESSEE: Incyte Pharmaceuticals, Inc.
 - (B) STREET: 3174 Porter Drive
 - (C) CITY: Palo Alto
 - (D) STATE: CA
 - (E) COUNTRY: U.S.
 - (F) ZIP: 94303
- (v) COMPUTER READABLE FORM:
 - (A) MEDIUM TYPE: Diskette
 - (B) COMPUTER: IBM Compatible
 - (C) OPERATING SYSTEM: DOS
 - (D) SOFTWARE: FastSEQ Version 1.5
- (vi) CURRENT APPLICATION DATA:
 - (A) PCT APPLICATION NUMBER: To Be Assigned
 - (B) FILING DATE: Filed Herewith
 - (C) CLASSIFICATION:
- (vii) PRIOR APPLICATION DATA:
 - (A) APPLICATION SERIAL NUMBER: US 08/221,531
 - (B) FILING DATE: 02-FEB-1994
- (vii) PRIOR APPLICATION DATA:
 - (A) APPLICATION SERIAL NUMBER: US 08/602,208
 - (B) FILING DATE: 015-FEB-1996
- (viii) ATTORNEY/AGENT INFORMATION:
 - (A) NAME: Billings, Lucy J.
 - (B) REGISTRATION NUMBER: 36,749
 - (C) REFERENCE/DOCKET NUMBER: PF-0054 PCT
- (ix) TELECOMMUNICATION INFORMATION:
 - (A) TELEPHONE: 415-855-0555
 - (B) TELEFAX: 415-845-4166
 - (C) TELEX:

(2) INFORMATION FOR SEQ ID NO:1:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 745 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: cDNA
- (vii) IMMEDIATE SOURCE:
 - (A) LIBRARY: Myeloid Terminal

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

| | | | | | | |
|------------|------------|------------|------------|------------|------------|-----|
| CGGACTACCG | TGTTTCCG | CAACTTCTTG | GATTATCCTC | GCCAAGGACT | TTGNAATATA | 60 |
| TTTTTCCGCC | TTTTCTGGAA | GGATTTCGCT | GCTTCCCGAA | GGTCTTGGAC | GAGCGCTCTA | 120 |
| GCTCTGTGGG | AAGGTTTNGG | GCTCTCTGGC | TCGGATTTTG | GAATTTCTCC | CTGGGGACTG | 180 |
| CCGTGGAGCC | GCATCCACTG | TGGATTATAA | TTGCAACATG | ACGCTGGAAG | AGCTCGTGGC | 240 |
| GTGCGACAAC | GCGGCGCAGA | AGATGCAGAC | GGTGACCGCC | GCGGTGGAGG | AGCTTTTGGT | 300 |
| GGCCGCTCAG | CGCCAGGATC | GCCTCACAGT | GGGGGTGTAC | GAGTCGGCCA | AGTTGATGAA | 360 |
| TGTGGACCCA | GACAGCGTGG | TCCTCTGCCT | CTTGGCCATT | AACGAGGAGG | AGGAGGATGA | 420 |
| CATCGCCCTG | CAAATCCACT | TCACGCTCAT | CCAGTCCTTC | TCCTGTAACA | ACGACATCAA | 480 |
| CATCGTGCGG | GTTTCGGGCA | TGCAGCGCCT | GGCGCAGCTC | CTGGGAGAGC | CGGCCGAGAC | 540 |
| CCAGGGCACC | ACCGAGGCC | GAGACCTGCA | TTGTCTCCTG | GTCACGAACC | CTCACACGGA | 600 |
| CGCCCGGAAG | AGCCACGGCT | TGGTGGAGGT | GGCCAGCTAC | TGCGAAGAAA | GCCGGGGCAA | 660 |
| CAACCACTGG | GTCCCTACA | TCTCTCTTCA | GGAACGCTGA | GGCCTTCCCA | GCAGCAGAAT | 720 |
| CTGTTTGAGT | TGCTGCCACA | ACCAA | | | | 745 |

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 160 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(vii) IMMEDIATE SOURCE:

- (A) LIBRARY: Myeloid Terminal

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

| | | | | | | | | | | | | | | | |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Met | Thr | Leu | Glu | Glu | Leu | Val | Ala | Cys | Asp | Asn | Ala | Ala | Gln | Lys | Met |
| 1 | | | 5 | | | | | | 10 | | | | | 15 | |
| Gln | Thr | Val | Thr | Ala | Ala | Val | Glu | Glu | Leu | Leu | Val | Ala | Ala | Gln | Arg |
| | | 20 | | | | | | 25 | | | | 30 | | | |
| Gln | Asp | Arg | Leu | Thr | Val | Gly | Val | Tyr | Glu | Ser | Ala | Lys | Leu | Met | Asn |
| | | 35 | | | | 40 | | | | | | 45 | | | |
| Val | Asp | Pro | Asp | Ser | Val | Val | Leu | Cys | Leu | Leu | Ala | Ile | Asn | Glu | Glu |
| | 50 | | | | | 55 | | | | | 60 | | | | |
| Glu | Glu | Asp | Asp | Ile | Ala | Leu | Gln | Ile | His | Phe | Thr | Leu | Ile | Gln | Ser |
| 65 | | | | 70 | | | | | 75 | | | | | 80 | |
| Phe | Ser | Cys | Asn | Asn | Asp | Ile | Asn | Ile | Val | Arg | Val | Ser | Gly | Met | Gln |
| | | | 85 | | | | | | 90 | | | | | 95 | |
| Arg | Leu | Ala | Gln | Leu | Leu | Gly | Glu | Pro | Ala | Glu | Thr | Gln | Gly | Thr | Thr |
| | | 100 | | | | | 105 | | | | | 110 | | | |
| Glu | Ala | Arg | Asp | Leu | His | Cys | Leu | Leu | Val | Thr | Asn | Pro | His | Thr | Asp |
| | 115 | | | | | 120 | | | | | 125 | | | | |
| Ala | Arg | Lys | Ser | His | Gly | Leu | Val | Glu | Val | Ala | Ser | Tyr | Cys | Glu | Glu |
| | 130 | | | | 135 | | | | | 140 | | | | | |
| Ser | Arg | Gly | Asn | Asn | Gln | Trp | Val | Pro | Tyr | Ile | Ser | Leu | Gln | Glu | Arg |
| 145 | | | | 150 | | | | | 155 | | | | | 160 | |

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 165 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(vii) IMMEDIATE SOURCE:

(A) LIBRARY: Myeloid Terminal

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

```

Met Thr Leu Glu Glu Phe Ser Ala Gly Glu Gln Lys Thr Glu Arg Met
 1           5           10           15
Asp Lys Val Gly Asp Ala Leu Glu Glu Val Leu Ser Lys Ala Leu Ser
      20           25           30
Gln Arg Thr Ile Thr Val Gly Val Tyr Glu Ala Ala Lys Leu Leu Asn
      35           40           45
Val Asp Pro Asp Asn Val Val Leu Cys Leu Leu Ala Ala Asp Glu Asp
      50           55           60
Asp Asp Arg Asp Val Ala Leu Gln Ile His Phe Thr Leu Ile Gln Ala
      65           70           75           80
Phe Cys Cys Glu Asn Asp Ile Asn Ile Leu Arg Val Ser Asn Pro Gly
      85           90           95
Arg Leu Ala Glu Leu Leu Leu Glu Thr Asp Ala Gly Pro Ala Ala
      100          105          110
Ser Glu Gly Ala Glu Gln Pro Pro Asp Leu His Cys Val Leu Val Thr
      115          120          125
Asn Pro His Ser Ser Gln Trp Lys Asp Pro Ala Leu Ser Gln Leu Ile
      130          135          140
Cys Phe Cys Arg Glu Ser Arg Tyr Met Asp Gln Trp Val Pro Val Ile
      145          150          155          160
Asn Leu Pro Glu Arg
      165

```

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 160 amino acids
 (B) TYPE: amino acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(vii) IMMEDIATE SOURCE:

(A) LIBRARY: Myeloid Terminal

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

```

Met Thr Leu Glu Glu Leu Val Ala Ser Asp Asn Ala Val Gln Lys Met
 1           5           10           15
Gln Ala Val Thr Ala Ala Val Glu Gln Leu Leu Val Ala Ala Gln Arg
      20           25           30
Gln Asp Arg Leu Thr Val Gly Val Tyr Glu Ala Ala Lys Leu Met Asn
      35           40           45
Val Asp Pro Asp Ser Val Val Leu Cys Leu Leu Ala Ile Asp Glu Glu
      50           55           60
Glu Glu Asp Asp Ile Ala Leu Gln Ile His Phe Thr Leu Ile Gln Ser
      65           70           75           80
Phe Cys Cys Asp Asn Asp Ile Asp Ile Val Arg Val Ser Gly Met Gln
      85           90           95
Arg Leu Ala Gln Leu Leu Gly Glu Pro Ala Glu Thr Leu Gly Thr Thr
      100          105          110
Glu Ala Arg Asp Leu His Cys Leu Leu Val Thr Asn Cys His Thr Asp
      115          120          125
Ser Trp Lys Ser Gln Gly Leu Val Glu Val Ala Ser Tyr Cys Glu Glu

```

| | | | | | | | | | | | | | | | |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| | 130 | | | | | 135 | | | | | 140 | | | | |
| Ser | Arg | Gly | Asn | Asn | Gln | Trp | Val | Pro | Tyr | Ile | Ser | Leu | Glu | Glu | Arg |
| 145 | | | | | 150 | | | | | 155 | | | | | 160 |

CLAIMS

1. A purified polynucleotide comprising a nucleic acid sequence encoding the polypeptide
5 having the sequence shown in SEQ ID NO: 2.
2. The purified polynucleotide of claim 1 wherein the nucleic acid sequence comprises the sequence shown in SEQ ID NO:1.
3. An antisense molecule comprising the complement of the polynucleotide of claim 1 or a portion thereof.
- 10 4. An expression vector comprising the polynucleotide of claim 1.
5. A host cell transformed with the expression vector of claim 4.
6. A method for producing the polypeptide having the amino acid sequence for MYD118 (SEQ ID NO:2), said method comprising the steps of:
 - a) culturing the host cell of claim 5 under conditions suitable for the expression of
15 said polypeptide, and
 - b) recovering said polypeptide from the host cell culture.
7. A purified polypeptide comprising the amino acid sequence for MYD118 as shown in SEQ ID NO:2.
8. A diagnostic composition for the detection of myd118 polynucleotide sequences
20 comprising the polynucleotide of claim 1.
9. A diagnostic test for the detection of myd118 polynucleotide sequences in a biological sample, comprising the steps of:
 - a) combining the biological sample with a first nucleotide sequence of SEQ ID NO: 1, or a non-conserved fragment thereof, under conditions suitable for the formation of a
25 nucleic acid hybridization complex,
 - b) detecting said hybridization complex, wherein the presence of said complex correlates with the presence of a second nucleotide sequence comprising myd118 polynucleotide sequences in said biological sample, and
 - c) comparing the amount of the second nucleotide sequence in said sample with a
30 standard thereby determining whether the amount of said second nucleotide sequence varies from said standard, wherein the presence of an abnormal level of said second nucleotide sequence correlates positively with a myeloproliferative disease.

10. A diagnostic test for the detection of myd118 polynucleotide sequences in a biological sample, comprising the steps of:
- a) combining the biological sample with polymerase chain reaction primer under conditions suitable for nucleic acid amplification, wherein said primers comprise non-conserved fragments of the nucleotide sequence of SEQ ID NO:1,
 - b) detecting amplified nucleotide sequences, and
 - c) comparing the amount of amplified nucleotide sequences in said biological sample with a standard thereby determining whether the amount of said nucleotide sequence varies from said standard, wherein the presence of an abnormal level of said nucleotide sequence correlates positively with a myeloproliferative disease.
11. A method of screening a plurality of compounds for specific binding affinity with the polypeptide of claim 7 or a portion thereof comprising the steps of:
- a) providing a plurality of compounds;
 - b) combining the polypeptide of claim 7 with each of a plurality of compounds for a time sufficient to allow binding under suitable conditions; and
 - c) detecting binding of said polypeptide of claim 7 to each of the plurality of compounds, thereby identifying the compounds which specifically bind said polypeptide of claim 7.
12. A method for inducing terminal differentiation of human proliferating myeloid cells comprising administering an effective amount of the polynucleotide of claim 1 to said myeloid cells under conditions suitable for expression of MYD118 (SEQ ID NO:2).
13. A method for inducing terminal differentiation of human proliferating myeloid cells comprising administering an effective amount of the polypeptide of claim 7 to said myeloid cells.
14. A method for inhibiting the expression of MYD118 in human hematopoietic cells comprising administering an effective amount of the antisense molecule of claim 3 to said cells.
15. The method of claim 14 wherein said hematopoietic cells are in vitro.
16. A pharmaceutical composition for treating an individual having a myeloproliferative disease comprising an effective amount of the polynucleotide of claim 1, or a biologically active fragment thereof.
17. A method of treating an individual having a myeloproliferative disease comprising

administering an effective amount of the pharmaceutical composition of claim 16 to said individual.

18. A pharmaceutical composition for treating an individual having a myeloproliferative disease comprising an effective amount of the polypeptide of claim 7, or a biologically
5 active fragment thereof.
19. A method of treating an individual having a myeloproliferative disease comprising administering an effective amount of the pharmaceutical composition of claim 18 to said individual.
20. An antibody specific for the polypeptide of claim 7.

```

          9       18       27       36       45       54
5'   C GGA CTA CCG TTG GTT TCC GCA ACT TCT TGG ATT ATC CTC GCC AAG GAC TTT

          63       72       81       90       99       108
      GNA ATA TAT TTT TCC GCC TTT TCT GGA AGG ATT TCG CTG CTT CCC GAA GGT CTT

          117      126      135      144      153      162
      GGA CGA GCG CTC TAG CTC TGT GGG AAG GTT TNG GGC TCT CTG GCT CGG ATT TTG

          171      180      189      198      207      216
      GAA TTT CTC CCT GGG GAC TGC CGT GGA GCC GCA TCC ACT GTG GAT TAT AAT TGC

          225      234      243      252      261      270
      AAC ATG ACG CTG GAA GAG CTC GTG GCG TGC GAC AAC GCG GCG CAG AAG ATG CAG
      M   T   L   E   E   L   V   A   C   D   N   A   A   Q   K   M   Q

          279      288      297      306      315      324
      ACG GTG ACC GCC GCG GTG GAG GAG CTT TTG GTG GCC GCT CAG CGC CAG GAT CGC
      T   V   T   A   A   V   E   E   L   L   V   A   A   Q   R   Q   D   R

          333      342      351      360      369      378
      CTC ACA GTG GGG GTG TAC GAG TCG GCC AAG TTG ATG AAT GTG GAC CCA GAC AGC
      L   T   V   G   V   Y   E   S   A   K   L   M   N   V   D   P   D   S

          387      396      405      414      423      432
      GTG GTC CTC TGC CTC TTG GCC ATT AAC GAG GAG GAG GAG GAT GAC ATC GCC CTG
      V   V   L   C   L   L   A   I   N   E   E   E   E   D   D   I   A   L

          441      450      459      468      477      486
      CAA ATC CAC TTC ACG CTC ATC CAG TCC TTC TCC TGT AAC AAC GAC ATC AAC ATC
      Q   I   H   F   T   L   I   Q   S   F   S   C   N   N   D   I   N   I

          495      504      513      522      531      540
      GTG CGG GTT TCG GGC ATG CAG CGC CTG GCG CAG CTC CTG GGA GAG CCG GCC GAG
      V   R   V   S   G   M   Q   R   L   A   Q   L   L   G   E   P   A   E

          549      558      567      576      585      594
      ACC CAG GGC ACC ACC GAG GCC CGA GAC CTG CAT TGT CTC CTG GTC ACG AAC CCT
      T   Q   G   T   T   E   A   R   D   L   H   C   L   L   V   T   N   P

          603      612      621      630      639      648
      CAC ACG GAC GCC CGG AAG AGC CAC GGC TTG GTG GAG GTG GCC AGC TAC TGC GAA
      H   T   D   A   R   K   S   H   G   L   V   E   V   A   S   Y   C   E

          657      666      675      684      693      702
      GAA AGC CGG GGC AAC AAC CAG TGG GTC CCC TAC ATC TCT CTT CAG GAA CGC TGA
      E   S   R   G   N   N   Q   W   V   P   Y   I   S   L   Q   E   R

          711      720      729      738      747
      GGC CTT CCC AGC AGC AGA ATC TGT TTG AGT TGC TGC CAC AAC CAA 3'

```

FIGURE 1

| | | | | | | |
|-----|--------------|------------|----------|---------|-----------|--|
| 1 | MTLEELVAC | 10 | 20 | 30 | 40 | |
| 1 | MTLEELVAS | DNAA | QKMQ | VTAAVEE | LLVAAQRQ | DRRLTVGV |
| 1 | MTLEELVFS | AGEQKTER | MDKVGDA | LEEVL | SKAL | SLORTITVG |
| | | | | | | 25214 cDNA#1.AMI MMVD118 HUNGADD45 |
| 41 | YESAKLMNVDP | 50 | 60 | 70 | 80 | |
| 41 | YEAALKLMNVDP | DSVVLCLLAI | NEEEEEED | DDIALQ | IIHFTLIQS | |
| 41 | YEAALKLMNVDP | DSVVLCLLAI | DEEEEEED | DDIALQ | IIHFTLIQS | |
| | | | | | | 25214 cDNA#1.AMI MMVD118 HUNGADD45 |
| 81 | FSCNNDINIV | 90 | 100 | 110 | 120 | |
| 81 | FCCDNDIIV | RVSGMQRLA | QLL--- | GEPAET | QGTTEAR | |
| 81 | FCCENDINI | LRVSNPGR | LAELLL | ETDAGPA | ASEGAEQPP | |
| | | | | | | 25214 cDNA#1.AMI MMVD118 HUNGADD45 |
| 116 | DLHCLLV | 130 | 140 | 150 | 160 | |
| 116 | DLHCLLV | TNPHTD | ARKSHGL | VEVAS | YCEESR | GNNQWVPYI |
| 121 | DLHCLLV | TNPHTD | SWKSKGL | VEVAS | YCEESR | GNNQWVPYI |
| | | | | | | 25214 cDNA#1.AMI MMVD118 HUNGADD45 |
| 156 | SLQER | | | | | |
| 156 | SLQER | | | | | |
| 161 | NLPER | | | | | |

FIGURE 2

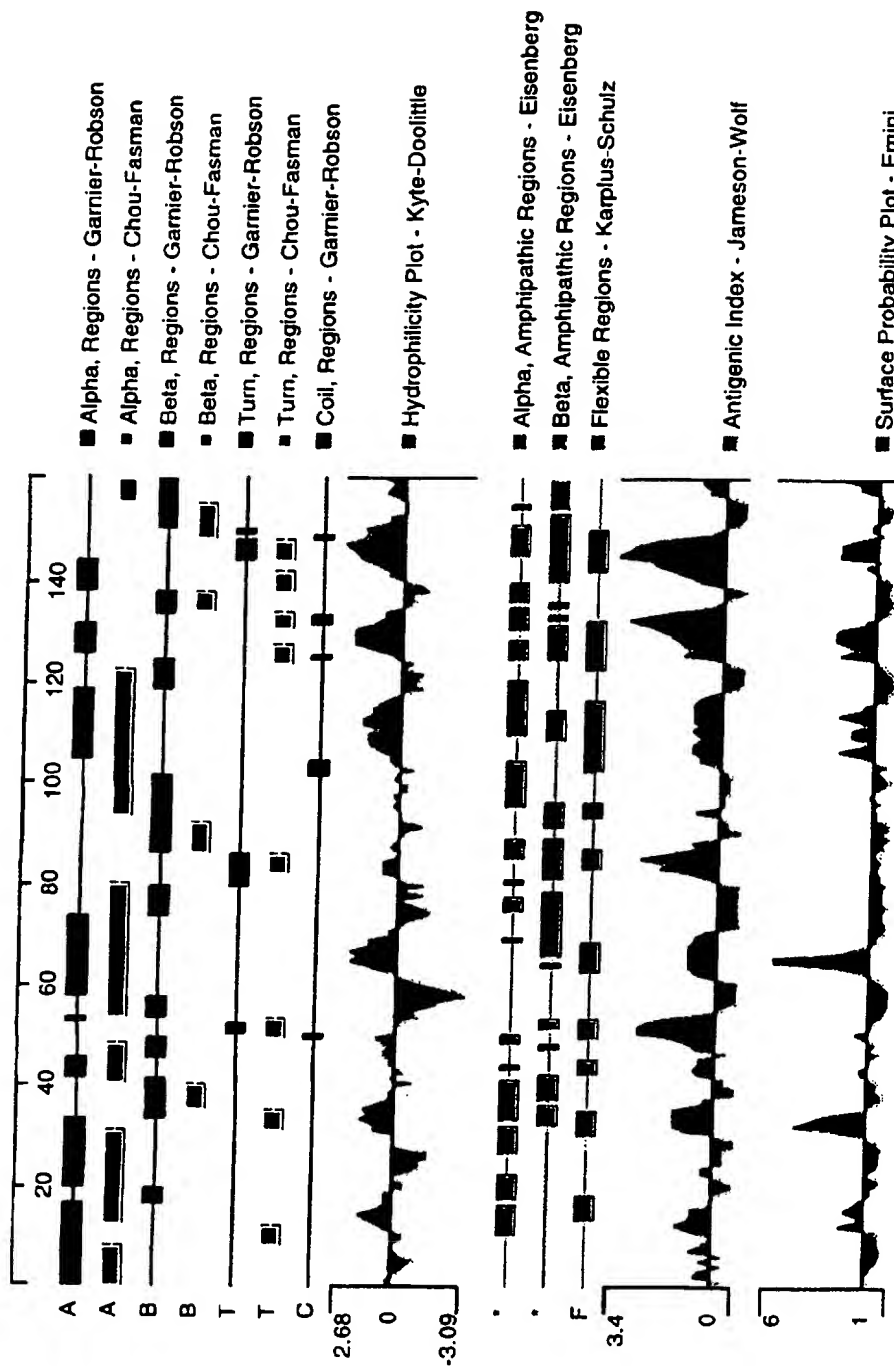


FIGURE 3

BEST AVAILABLE COPY

INTERNATIONAL SEARCH REPORT

International application No

PCT/US 97/02458

A. CLASSIFICATION OF SUBJECT MATTER

| | | | | | |
|-------|-----------|-----------|-----------|-----------|-----------|
| IPC 6 | C12N15/19 | C12N15/11 | C12N15/85 | C12N5/10 | C12Q1/68 |
| | C07K16/24 | A61K38/19 | A61K48/00 | G01N33/53 | C07K14/47 |

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 6 C07K C12N C12Q A61K G01N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

| Category | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|----------|--|-----------------------|
| X | EMBL DATABASE, 9 January 1995, HEIDELBERG, XP002033953 ADAMS M. ET AL.: "AC T29941" cited in the application see the whole document --- | 1,2 |
| X | EMBL DATABASE, 22 February 1992, HEIDELBERG, XP002033954 ADAMS M. ET AL.: "AC M77995" cited in the application see the whole document --- | 1,2 |
| Y | --- | 3-6,12, 14,15 |
| | -/-- | |

☒ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

* Special categories of cited documents:

- * "A" document defining the general state of the art which is not considered to be of particular relevance
- * "E" earlier document but published on or after the international filing date
- * "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- * "O" document referring to an oral disclosure, use, exhibition or other means
- * "P" document published prior to the international filing date but later than the priority date claimed

- * "T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
- * "X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
- * "Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.
- * "&" document member of the same patent family

| | |
|--|--|
| Date of the actual completion of the international search | Date of mailing of the international search report |
| 30 June 1997 | 16.07.97 |
| Name and mailing address of the ISA European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+ 31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+ 31-70) 340-3016 | Authorized officer Kania, T |

INTERNATIONAL SEARCH REPORT

International Application No
PCT/US 97/02458

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

| Category * | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|------------|--|-----------------------|
| Y | <p>MOLECULAR AND CELLULAR BIOLOGY, vol. 14, no. 4, April 1994, pages 2352-2360, XP000676663 SELVAKUMARAN M. ET AL.: "The novel primary response gene MyD118 and the protooncogenes myb, myc, and bcl-2 modulate transforming growth factor beta1-induced apoptosis of myeloid leukemia cells" cited in the application see the whole document</p> <p style="text-align: center;">---</p> | 3-6,14, 15 |
| Y | <p>MOLECULAR AND CELLULAR BIOLOGY, vol. 14, no. 4, April 1994, pages 2361-2371, XP000676678 ZHAN Q. ET AL.: "The gadd and MyD genes define a novel set of mammalian genes encoding acidic proteins that synergistically suppress cell growth" cited in the application see the whole document</p> <p style="text-align: center;">---</p> | 12 |
| A | <p>WO 94 11533 A (US HEALTH ;FORNACE ALBERT J JR (US); KASTAN MICHAEL B (US); CARRIE) 26 May 1994 see the whole document</p> <p style="text-align: center;">---</p> | 1-20 |
| A | <p>ONCOGENE, vol. 6, no. 1, January 1991, pages 165-167, XP000676665 ABDOLLAHI A. ET AL.: "Sequence and expression of a cDNA encoding MyD118: a novel myeloid differentiation primary response gene induced by multiple cytokines" cited in the application see the whole document</p> <p style="text-align: center;">---</p> | 1-5 |
| A | <p>EP 0 356 180 A (SCHERING CORP) 28 February 1990 see the whole document</p> <p style="text-align: center;">---</p> | 16-19 |
| A | <p>STEM CELLS, vol. 12, no. 4, 1994, pages 352-369, XP000676664 LIEBERMANN D. AND HOFFMAN B.: "Differentiation primary response genes and proto-oncogenes as positive and negative regulators of terminal hematopoietic cell differentiation" cited in the application * see esp. pp. 358-60 *</p> <p style="text-align: center;">---</p> | 1-20 |

-/--

INTERNATIONAL SEARCH REPORT

International Application No.

PCT/US 97/02458

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

| Category * | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|------------|---|-----------------------|
| P,X | EMBL DATABASE, 25 January 1997, HEIDELBERG, XP002033955 HILLIER L. ET AL.: "AC AA194908" see the whole document --- | 1,2 |
| T | ONCOGENE, vol. 12, no. 12, 20 June 1996, pages 2579-2594, XP000676666 VAIRAPANDI M. ET AL.: "The differentiation primary response gene MyD118, related to GADD45, encodes for a nuclear protein which interacts with PCNA and P21(WAF1/CIP1)" * see esp. p. 2587/88, p.2584-86 * ----- | 11,12 |

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 97/02458

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☒ Claims Nos.: 12-14, 17, 19
because they relate to subject matter not required to be searched by this Authority, namely:
Remark: Although claims 12-14, 17, 19 (as far as in vivo methods are concerned) are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.
2. ☐ Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:
3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest.

☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/US 97/02458

| Patent document cited in search report | Publication date | Patent family member(s) | Publication date |
|---|---------------------|--|----------------------------------|
| WO 9411533 A | 26-05-94 | AU 5605994 A US 5616463 A | 08-06-94 01-04-97 |
| EP 0356180 A | 28-02-90 | AU 4219089 A JP 4500208 T WO 9001943 A | 23-03-90 16-01-92 08-03-90 |